Synthesis and Biological Evaluation of Polyunsaturated Natural Products and Derivatives

Dissertation for the degree of Ph.D.

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"Success is the sum of small efforts, repeated day in and day out." Robert Collier

"Identify your problems but give your power and energy to solutions." Tony Robbins

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Yasser Mohamed

Oslo, January 2013

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Abstract

In recent years, marine natural products have gained attention from scientists within diverse research fields, such as biology, pharmacology, medicine and chemistry. Among those natural products, several polyunsaturated fatty acids (PUFAs) and their metabolites have been isolated.

This prompted us to develop an efficient synthesis of analogs of the two PUFA marine natural products mycalazol 5 and mycalazal 2 starting from eicosapentaenoic acid (EPA) and eicosanoic acid (EA). The biological studies revealed that all synthesized analogs displayed potent cytotoxic effect against several human cancer cell lines.

In continuation of this study, another PUFA derived marine natural product was used as a lead compound for making analogs that were subjected to biological testing as antioxidants. The analogs with a polyunsaturated alkyl chain exhibited more potent effects than the analogs possessing a saturated alkyl chain.

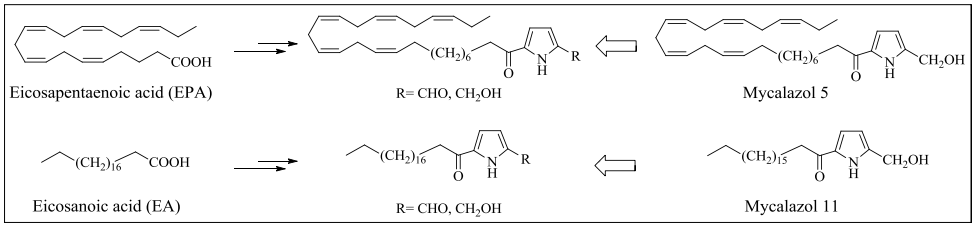
Bosseopentaenoic acid (BPA) and its methyl ester have been isolated from different algae. This PUFA contains a (*Z*,*E*,*E*,*Z*)-conjugated polyene moiety with five double bonds in total. An efficient stereocontrolled synthesis of this natural product has been achieved.

During the work towards the total synthesis of BPA, a *Z*-stereoselective Boland reduction reaction was needed. The Boland reduction procedure was modified by the addition of TMSCl, which resulted in shorter reaction times. This procedure was applied for the preparation of several *Z*-alkenes.

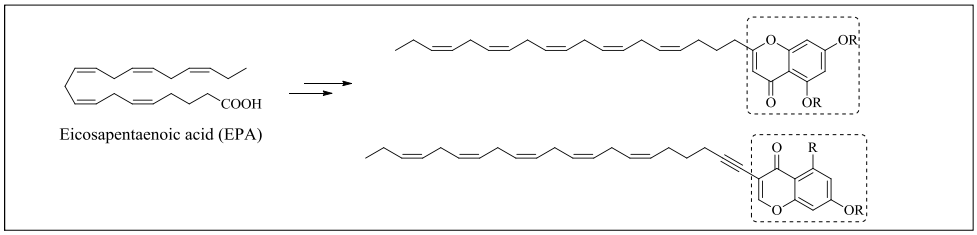
As a continuation of the synthetic work towards BPA, the synthesis of α-parinaric acid was also investigated. As in the case with BPA, this naturally occurring PUFA also contains a *Z*,*E*,*E*,*Z*-conjugated polyene moiety, that we attempted to prepare *via* iterative palladium-catalyzed cross coupling reactions.

Graphical Abstract

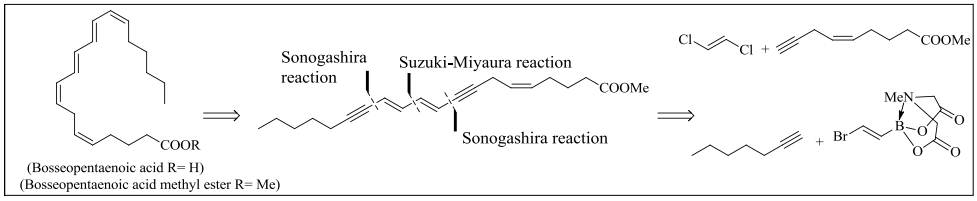
Synthesis of mycalazol and mycalazal analogs with potent antiproliferating activities.



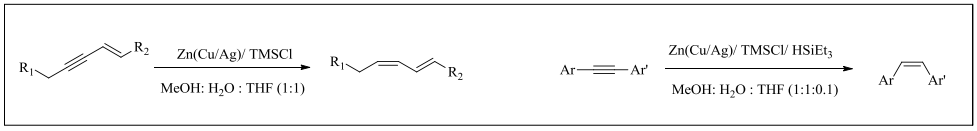
Polyunsaturated fatty acid-derived chromones exhibiting potent antioxidant activity.



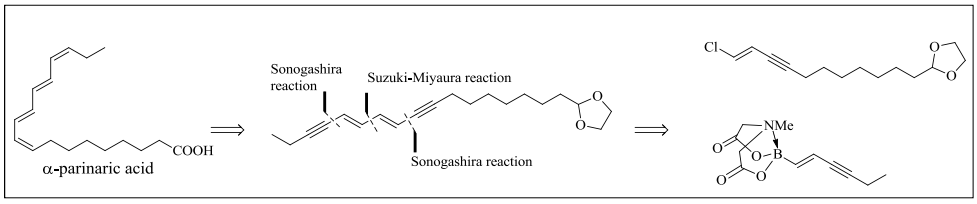
First total synthesis of methyl (5*Z*,8*Z*,10*E*,12*E*,14*Z*)-eicosapentaenoate.



***Z*-Stereoselective semi-reduction of alkynes: Modification of the Boland protocol.**



Synthetic studies towards α-parinaric acid.



List of Abbreviations

AA	Arachidonic acid
ALA	α-linolenic acid
BPA	Bosseopentaenoic acid
CDHA	Conjugated docosahexaenoic acid
CEPA	Conjugated eicosapentaenoic acid
CLA	Conjugated linoleic acid
CLPAA	Cellular Lipid Peroxidation Antioxidant Activity
COX	Cyclooxygenase
DBU	1,8-Diazabicycloundec-7-ene
DIBAl-H	Diisobutyl aluminium hydride
DHA	Docosahexaenoic acid
DMFMFA	Dimethylformamide-dimethyl acetal
DMSO	Dimethyl sulfoxide
dppf	1,1'-Bis(diphenylphosphino)ferrocene
EA	Eicosanoic acid
EPA	Eicosapentaenoic
GLC	Gas liquid chromatography
HETE	Hydroxy eicosatetraenoic acid
HepG2	Human hepatoma cell line
HMPA	Hexamethylphosphoramide
HPLC	High performance liquid chromatography
HPETE	Hydro-peroxy-eicosatetraenoic acid
ICC	Iterative cross coupling
IC_{50}	Concentration that inhibits 50% of a given biological process
LA	Linoleic acid
LDA	Lithium diisopropyl amine
LO	Lipoxygenase
MIDA	*N*-methyliminodiacetic acid
MOMBr	Methoxy methyl bromide
n-BuLi	*n*-Butyl lithium
NaHMDS	Sodium bis(trimethylsilyl)amide

PTSA	*p*-Toluenesulfonic acid
ppm	Parts per million
PUFAs	Polyunsaturated fatty acids
ROS	Reactive oxygen species
rt	Room temperature
SPhos	2-Dicyclohexylphosphino-2′,6′-dimethoxybiphenyl
THF	Tetrahydrofuran
THP	Tetrahydopyran
TMS	Trimethylsilyl
TMSCl	Trimethysilyl chloride
Ts	Tosyl
X-Phos	2-Dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl
Δ	reflux

List of publications

This thesis is based on the following publications

Paper I

Synthesis of mycalazol and mycalazal analogs with potent antiproliferating activities.

Mohamed, Y. M. A.; Hansen T. V. *Pure and Applied Chemistry* **2011**, *83*, 489-493.

Paper II

Polyunsaturated fatty acid-derived chromones exhibiting potent antioxidant activity.

Mohamed, Y. M. A.; Vik, A.; Hofer, T.; Andersen, J. H.; Hansen, T. V. *Chem. Phys. Lipids* **2013**, submitted.

Paper III

First total synthesis of methyl (5*Z*,8*Z*,10*E*,12*E*,14*Z*)-eicosapentaenoate.

Mohamed Y. M. A.; Hansen T. V. *Tetrahedron Lett*. **2011**, *52*, 1057-1059.

Paper IV

Z-Stereoselective semi-reduction of alkynes: Modification of the Boland protocol.

Mohamed, Y. M. A.; Hansen, T. V. *Tetrahedron* **2013**, *69*, under revision.

1. Introduction

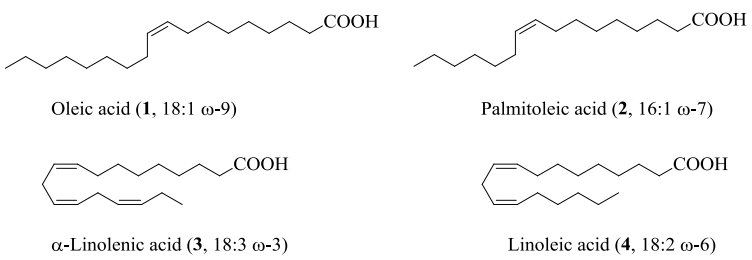
1.1. Lipids and fatty acids

The natural products within the class of lipids constitute a wide range of compounds with diverse chemical structures. Traditionally, lipids have been defined as organic compounds that are soluble in a lipophilic solvent, such as chloroform or ether.[1] Recently, however, lipids have been defined as "hydrophobic or amphipathic small molecules that may originate entirely or in part by carbanion based condensations of thioesters and/or by carbocation-based condensations of isoprene units".[2] Among the eight classes of naturally occurring compounds defined as lipids according to this new definition, the class of fatty acyls or fatty acids is a central one.[1] Chemically, fatty acids are hydrocarbons attached to a carboxylic acid which possess a long aliphatic chain which is either saturated or unsaturated.[3]

Among the many unsaturated fatty acids found in nature, the ω-3 and ω-6 polyunsaturated fatty acids (PUFAs) have received a great interest from scientists in biology, medicine and chemistry.[4]

1.2. ω-3 and ω-6 polyunsaturated fatty acids (PUFAs)

PUFAs are common in higher organisms and exhibit interesting biological activities. In particular, the PUFAs are characterized by the presence of several methylene interrupted double bonds, *i.e.* those with two or more *cis*-configured double bonds isolated by a single methylene group. PUFAs have received a lot of interest over the last fifty years.[5,6,7] There are four families of polyunsaturated fatty acids, namely ω-9, ω-7, ω-6 and ω-3 PUFAs.[8] Oleic acid (**1**) is the precursor of the ω-9 family and palmitoleic acid (**2**) is the precursor of the ω-7 family.



Oleic acid (**1**, 18:1 ω-9) Palmitoleic acid (**2**, 16:1 ω-7)

α-Linolenic acid (**3**, 18:3 ω-3) Linoleic acid (**4**, 18:2 ω-6)

Figure 1.1. The chemical structures of the primary precursors molecules for the PUFA families.

Oleic acid (**1**) and palmitoleic acid (**2**) are non-essential fatty acids that are synthesized in the human body from dietary precursors. The two principal families of polyunsaturated fatty acids that occur in nature are derived biosynthetically from α-linolenic acid (**3**, 9*Z*,12*Z*,15*Z*-octadecatrienoic acid) and linoleic acid (**4**, 9*Z*,12*Z*-octadecadienoic acid). The acids **3** and **4** are the primary precursor molecules for the ω-3 and ω-6 families, respectively.[9]

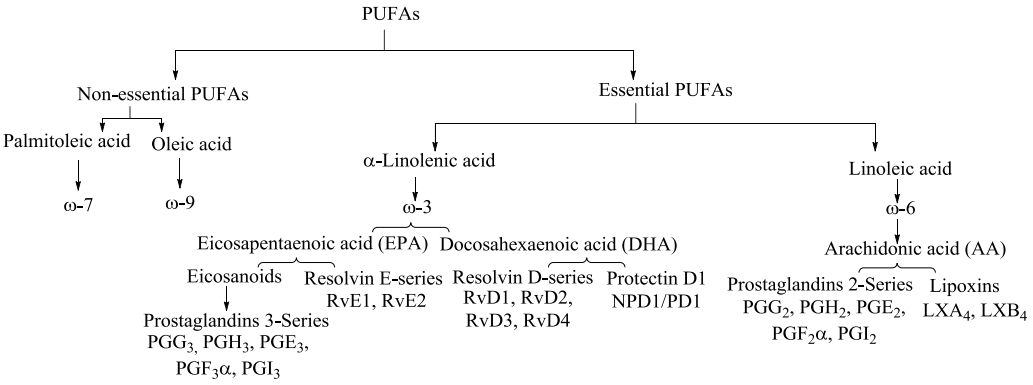
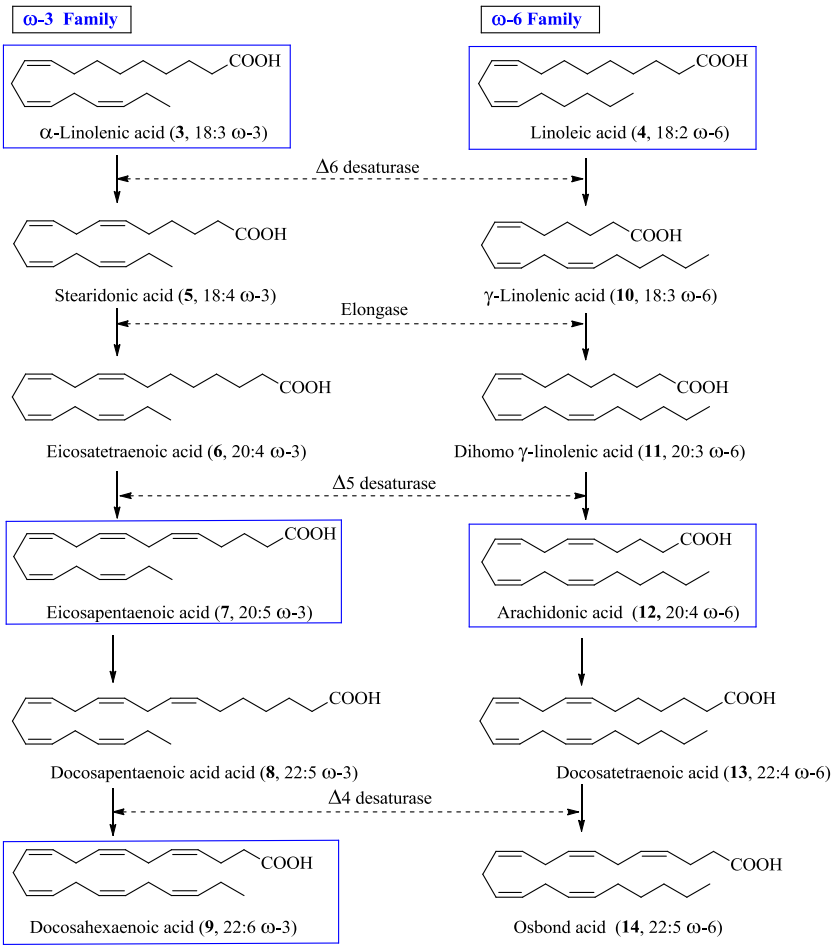


Figure 1.2. An overview of the PUFAs families.

α-Linolenic (ALA, **3**) and linoleic (LA, **4**) acids are converted to their respective metabolites by a series of alternating desaturation and elongation reactions.[10,11] The desaturation reaction adds one double bond by removing two atoms of hydrogen, while the elongation reaction adds two carbon atoms. The main pathway to the formation of docosahexaenoic acid (DHA, **9**, 22:6, ω-3) requires a sequence of chain elongation and desaturation steps (Δ5 and Δ6 desaturases with acyl-coenzyme-A esters as substrates)[12,13] as illustrated in Scheme 1.1. Thus, α-linolenic (**3**) is sequentially elongated and desaturated with double bonds being inserted between existing double bonds and carboxyl group to form eicosapetaenoic acid (EPA, **7**, 20:5, ω-3) which is the precursor of DHA (**9**) (Scheme 1.1).[14] Linoleic acid (**4**) is the precursor for γ-linolenic acid (**10**, 18:3, ω-6). The first step involves desaturation with introduction of a double bond in position 6 to form γ-linolenic acid (**10**). Chain elongation by a two carbon unit gives dihomo γ-linolenic acid (**11**, 20:3, ω-6), which is converted to arachidonic acid (**12**) by Δ5 desaturase. Arachidonic acid (**12**) is a metabolite of linoleic acid (**4**) and is considered an essential fatty acid only when linoleic acid deficiency exists.[15] However, two further chain elongation steps yield first docosatetraenoic acid (**13**, 22:4, ω-

6),[16] which can be further desaturated by a Δ6 desaturase to osbond acid (**14**, 22:5, ω-6) (Scheme 1.1).[17]

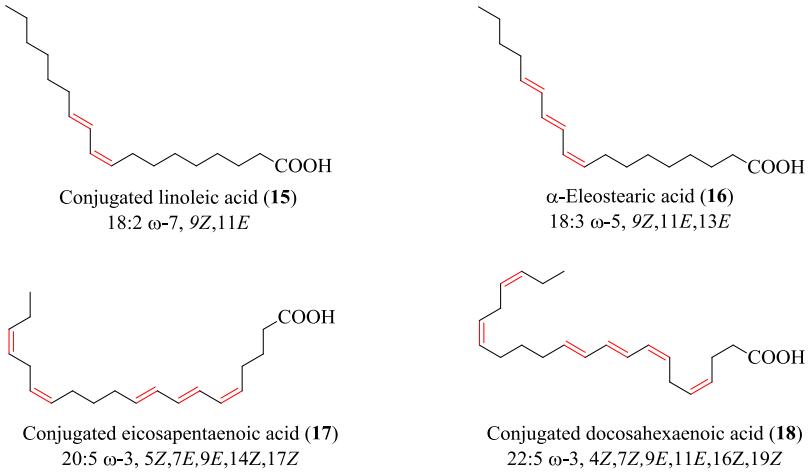


Scheme 1.1.The elongation and desaturation of linoleic acid (**3**) and α-linolenic acid (**4**) to PUFAs.

1.3. Conjugated polyunsaturated fatty acids

Conjugated fatty acids are polyunsaturated fatty acids in which at least one pair of double bonds are separated only by one single bond, as in conjugated linoleic acid (CLA, **15**), (Figure 1.3). Conjugated linoleic acid has been reported to have many beneficial medical effects,[18-22] especially anticancer activity.[23-30] Fatty acids with a conjugated triene systems have been found in a large number of different plant species. α-Eleostearic acid (**16**, 18:3, ω-

5), conjugated EPA (CEPA, **17**, 20:5, ω-3) and conjugated DHA (CDHA, **18**, 22:5, ω-3) are the most widespread and best known.[31,32]



Conjugated linoleic acid (**15**)
18:2 ω-7, *9Z*,11*E*

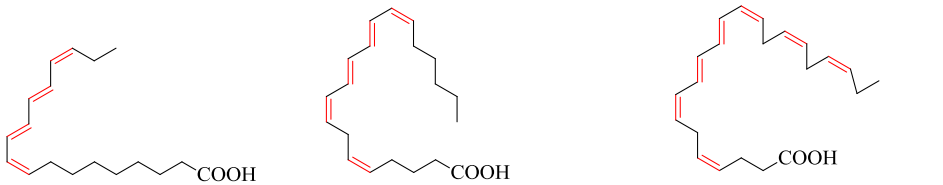
α-Eleostearic acid (**16**)
18:3 ω-5, *9Z*,11*E*,13*E*

Conjugated eicosapentaenoic acid (**17**)
20:5 ω-3, 5*Z*,7*E*,9*E*,14Z,17*Z*

Conjugated docosahexaenoic acid (**18**)
22:5 ω-3, 4*Z*,7*Z*,9*E*,11*E*,16Z,19*Z*

Figure 1.3. Conjugated diene and triene polyunsaturated fatty acids.

In 1933, Tsujimoto and Koyanagi isolated α-parinaric acid (**19**,18:4, ω-3) from the seed oil of *Parinarium laurinum*.[33] α-Parinaric acid (**19**) is a (*Z*,*E*,*E*,*Z*) conjugated tetraene fatty acid and is commonly used as a molecular probe in the study of biomembranes due to the fluorescent properties conferred by the alternating double bonds.[34]



α-parinaric acid (**19**)
18:4 ω-3, *9Z*,11*E*,*13E*,15*Z*

Bosseopentaenoic acid (**20**)
20:5 ω-3, 5*Z*,8*Z*,10*E*,12*E*,14*Z*

Stellaheptaenoic acid (**21**)
22:7 ω-3, 4*Z*,7*Z*,9*E*,11*E*,13*Z*,16Z,19*Z*

Figure 1.4. Conjugated tetraene polyunsaturated fatty acids.

Bosseopentaenoic acid (BPA, **20**, 5*Z*,8*Z*,10*E*,12*E*,14*Z*-eicosapentaenoic, 20:5, ω-6) is also a (*Z*,*E*,*E*,*Z*) conjugated tetraene fatty acid that has been isolated from several algea.[35-37] In 1995, Jacobs and co-workers[37] isolated stellaheptaenoic acid (**21**, 4*Z*,7*Z*,9*E*,11*E*,13*Z*,16*Z*,19*Z*-docosaheptaenoic acid, 22:7, ω-3) which is a unique (*Z*,*E*,*E*,*Z*) conjugated tetraene fatty acid with seven double bonds in total.

1.4. Biological role of ω-3 and ω-6 polyunsaturated fatty acids

The ω-3 and ω-6 PUFAs play an important role in health and treatment of diseases. They can act as antibacterial agents,[38-40] anti-inflammatory agents,[41-42] antioxidants,[43] in the treatment of cardiovascular diseases[44] and cancer cell proliferation.[45,46] Such properties are indicative of the potential for PUFAs as nutraceuticals and as pharmaceuticals. The (ω-3/ω-6) ratio can significantly influence the body's metabolic function,[47] where an increased intake of ω-3 PUFAs leads to the replacement of ω-6 PUFAs in cells and tissues resulting in a reduction of the overall ω-3/ω-6 ratio. Also a reduction of arachidonic acid (**12**) derived metabolites has been observed. Dietary intake of ω-3 fatty acids may prevent the development of disease. An excessive amounts of ω-6 PUFAs (a very high ω-6/ω-3 ratio) promote the pathogenesis of many diseases, including cardiovascular disease, cancer, as well as inflammatory and autoimmune diseases,[48-50] whereas increased levels of ω-3 PUFAs (a low ω-6/ω-3 ratio) exert suppressive effects. Clinical studies indicate that increased intake of ω-3 PUFAs appear to reduce mortality from cardiovascular disease. So it is essential to increase the ω-3 PUFAs and decrease the ω-6 PUFAs intake in order to reduce the risk of many of the chronic diseases.[49,50]

Eicosapentaenoic acid (EPA, **7**) and docosahexaenoic acid (DHA, **9**) have the most potent anti-inflammatory effects.[51] Inflammation is at the base of many chronic diseases, including cardiovascular disease, diabetes, arthritis, cancer, osteoporosis, mental health, dry eye disease and age-related macular degeneration (Figure 1.5).[52]

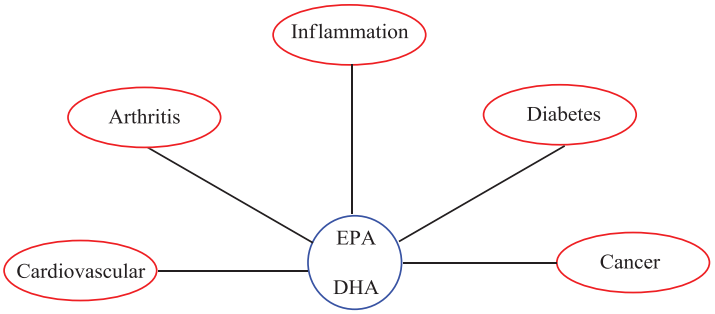


Figure 1.5. Health benefits of EPA and DHA.

Arachidonic acid (**12**) has long been known to be involved in the initiation involved in the biosynthesis of anti-inflammatory and pro-resolving lipid mediators. These include lipoxins

which are formed *via* transcellular biosynthesis through the sequential actions of 5- and 12-lipoxogenase.[53] EPA (**7**), DHA (**9**) and arachidonic acid (**12**) influence gene expression.[54]

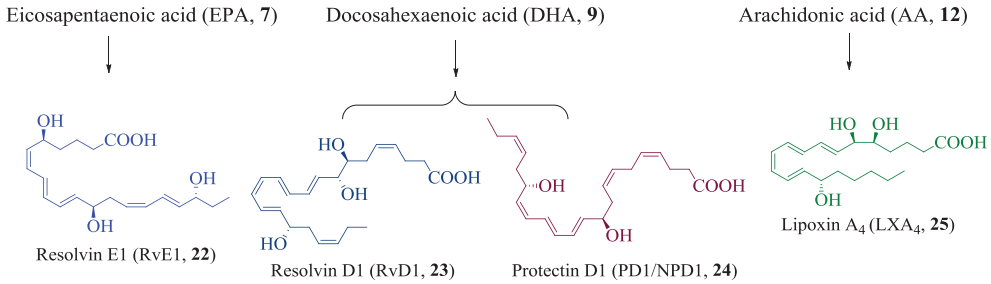


Figure 1.6. Examples of lipid mediators.

The enzymatic oxygenation of EPA and DHA has been investigated intensively by Serhan and co-workers.[55-57] These studies has led to the identification of novel pro-resolving lipid mediators. These include the E-series resolvins derived from EPA (RvE1, RvE2), as well as the D-series resolvins (RvD1, RvD2, RvD3, RvD4), the protectins (PD1) derived from DHA.[58]

1.5. Anticarcinogenic action of non-conjugated and conjugated fatty acids

The ω-3 PUFAs, *i.e.* α-linolenic acid (ALA, **3**), EPA (**7**) and DHA (**9**) induce *in vitro* and *in vivo* cytotoxic activity against different tumor cells.[59-61] Conjugated linoleic acid (CLA, **15**) has also been shown to inhibit the cell proliferation of different cancer cell lines.[23-30] However, recently numerous studies have shown that conjugated eicosapentaenoic acid (CEPA, **17**) and conjugated docosohexaenoic acid (CDHA, **18**) induces apoptosis *via* lipid peroxidation[62-66] which provides evidence that the conjugated triene polyunsaturated fatty acids (CEPA) and (CDHA) have the strongest cytotoxic effect (Figure 1.7). It was found that CEPA and CDHA caused apoptosis *via* formation of lipid peroxidation products *in vivo* that induce cytotoxic effect. This cytotoxic action were selective for tumor cells causing cell death only in tumor cells.[65,66]

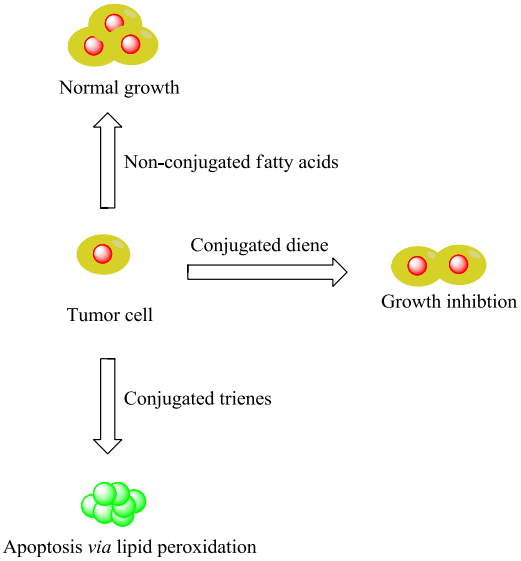


Figure 1.7. Anticarcinogenic action of non-conjugated and conjugated PUFAs.

It is possible that conjugated tetraenes such as bosseopentaenoic acid (BPA, **20**) and stellaheptaenoic acid **21** induce tumor cell death in the same manner as described for CEPA and CDHA.

1.6. Metabolism of ω-3 and ω-6 PUFAs

1.6.1. α-Cyclooxygenase and prostaglandins biosynthesis

In the late 1980 and 1990s a series of discoveries were made that led to the identification of two COX enzymes:[69-75] COX-1, which is a constitutively expressed isoform involved in physiologic maintenance functions, and COX-2, which is predominantly synthesized in response to inflammatory stimuli. The COX enzyme has two distinct active sites, respectively termed the cyclooxygenase active site and the peroxidase active site. The cyclooxygenase site cyclizes arachidonic acid (AA, **12)** and adds a hydroperoxy group to carbon 15 to form prostaglandin G_2 (PGG_2, **26**) (Figure 1.8). The separate peroxidase site of the same COX enzyme then reduces this hydroperoxy group to the hydroxy group to form prostaglandin H_2 (PGH_2, **27**) (Figure 1.8).[69-72] COX-1 and COX-2 have identical enzymatic actions, and the synthesis of PGH_2 is depending on the tissue where it is synthesized.[76]

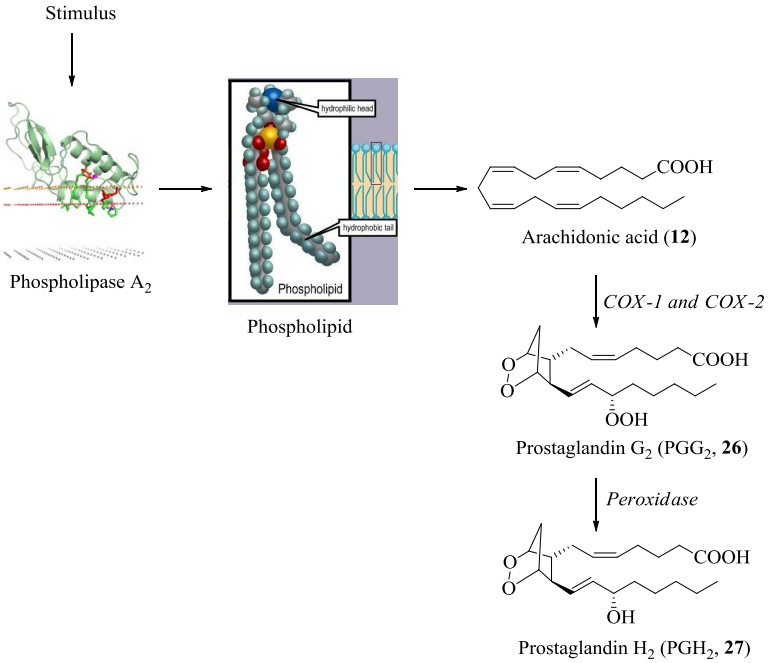


Figure 1.8. Biosynthetic pathway of prostaglandins.

COX-2-specific nonsteroidal anti-inflammatory drugs (NSAIDs) are lipophilic organic acids. Thus, the lower the pH, the greater is their lipophilicity. This combination of chemical properties allows the COX-2-specific NSAIDs (as well as conventional NSAIDs) to cross lipid membranes, including the blood-brain barrier, and to accumulate in acidic tissues such as the stomach, renal medulla, and sites of inflammation.[77] The COX-2 selectivity of NSAIDs is defined by the COX-2/COX-1 ratio.[78] The larger this ratio, the greater the selectivity of the compound is for COX-2. However, this ratio can vary significantly, depending on which of the different *in vitro* and *in vivo* assays that are used to generate the ratio.[78]

1.6.2. Lipoxyganse and leukotriene biosynthesis

Unlike the cyclooxygenases (COX-1 and COX-2), which are active in most body cells, lipoxygenase enzymes are primarily active in cells of the immune system. Lipoxygenases are enzymes that catalyse the stereospecific incorporation of molecular oxygen into polyunsaturated fatty acids.[79]

5-Lipoxygenase (5-LO) creates hydroperoxide PUFAs by an insertion of molecular oxygen.[80] Leukotriene biosynthesis depends upon the availability of arachidonic acid as a free carboxylic acid as the 5-LO substrate, which typically requires the action of cytosolic phospholipase A_2 to release arachidonic acid from membrane phospholipids.[81] The name leukotriene was conceived to capture two unique attributes of these molecules. The first attribute relates to those white blood cells derived from the bone marrow that have the capacity to synthesize this class of eicosanoid, for example the polymorph nuclear leukocyte. The last part of the name refers to the unique chemical structure, a conjugated triene retained within these eicosanoids.[82] The first step for the leukotriene biosynthesis is the insertion of molecular oxygen at position-5 of arachidonic acid (**12**) to produce 5(*S*)-hydroperoxy-eicosatetraenoic acid (5-HPETE, **28**) that can be converted to leukotriene A_4 (LTA_4, **30**) by the second catalytic activity of 5-lipoxyganse.[81]

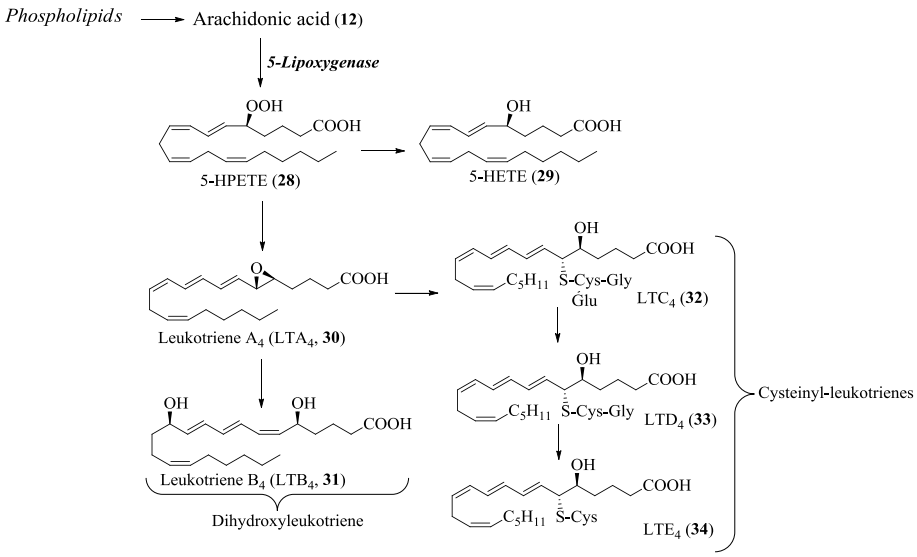


Figure 1.9. An overview of the biosynthetic pathway of leukotrienes.

Leukotriene A_4 is the precursor of leukotriene B_4 (LTB_4, **31**) including also leukotrienes (LTC_4, **32**), (LTD_4, **33**) and (LTE_4, **34**). Leukotriene B_4 is showing chemotactic and chemokinetic activity *in vivo*,[83] while LTC_4 exhibit potent smooth muscle contracting activity, and mediates leakage of vascular fluid in the process of edema.[84,85]

12-LO and 15-LO catalyze the transformation of free arachidonic acid (**12**) to 12(*S*)-hydroperoxy-5*Z*,8*Z*,9*E*,13*Z*-eicosatetraenoic acid (12-HPETE, **35**) and 15(*S*)-hydroperoxy-5*Z*,8*Z*,11*Z*,13*E*-eicosatetraenoic acid (15-HPETE, **37**). These products are reduced to the corresponding hydroxy derivatives 12-HETE **36** and 15-HETE **38** by cellular peroxidases. The first step of the biosynthesis of lipoxin A_4 (LXA_4, **25**) and lipoxin B_4 (LXB_4, **40**) is the formation of 15-HPETE **35** by a 15-lipoxygenase then reduced by a 5-lipoxygenase to form first an epoxy intermediate*, i.e.* 5*S*,6*S*-epoxy-15*S*-hydroxy-ETE (**39**) and then, depending on the cell type, by specific hydrolases to form either 5*S*,6*R*,15*S*-trihydroxy-7*E*,9*E*,13*E*,11*Z*-eicosatetraenoic acid (LXA_4, **25**), or to 5*S*,14*R*,15*S*-trihydroxy-6*E*,10*E*,12*E*,8*Z*-eicosa-tetraenoic acid (LXB_4, **40**) (Figure 1.10).[87] These bioactive trihydroxytetraene containing lipid mediators appear to function as stop signals for inflammatory responses and to promote repair and wound healing.[53]

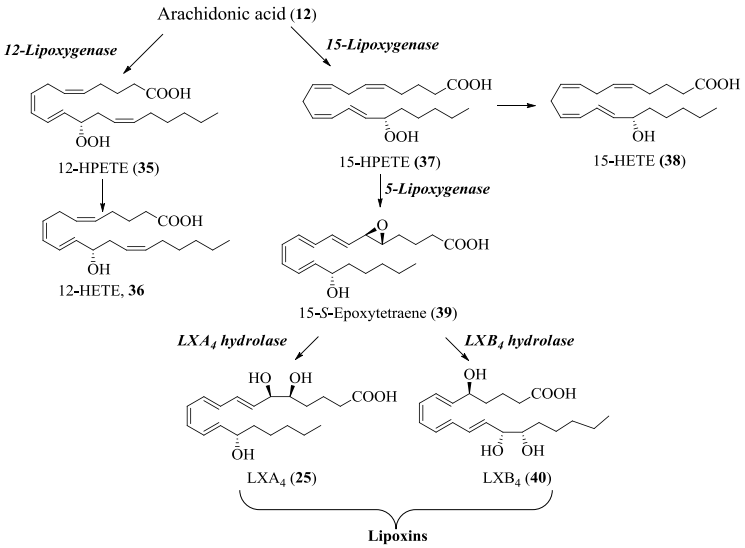


Figure 1.10. Catalytic action of 12/15 LO on arachidonic acid (**12**) and formation of lipoxins.

1.7. Some naturally occurring ω-3 and ω-6 PUFAs

1.7.1. Eicosanoids

The main eicosanoids are derived from the linoleic series (metabolites of arachidonic acid, AA) and of the α-linolenic series (metabolites of eicosapentaenoic acid). The eicosanoids

have traditionally been classified into four families (prostaglandins, prostacyclins, thromboxanes and leukotrienes).[88]

1.7.1.1. Prostaglandins

The prostaglandins are lipid mediators with physiological effects, such as regulation of the contraction and relaxation of smooth muscle tissue. They are synthesized in the cell from the essential fatty acids (EFAs). Bergström and Samuelsson isolated and structural elucidated the chemical structures of many prostaglandins.[89-93] Sir John Vane discovered that aspirin-like drugs could inhibit the synthesis of prostaglandins.[94-97] Bergström, Samuelsson and Vane received the 1982 Nobel Prize in Physiology or Medicine for their research on prostaglandins. E. J. Corey reported the first total syntheses of prostaglandin E_2 (**41**) and prostaglandin $F_{2\alpha}$ (**42**) in 1969.[98]

The prostaglandins of series-2 are derived from arachidonic acid (**12**) (Figure 1.11).

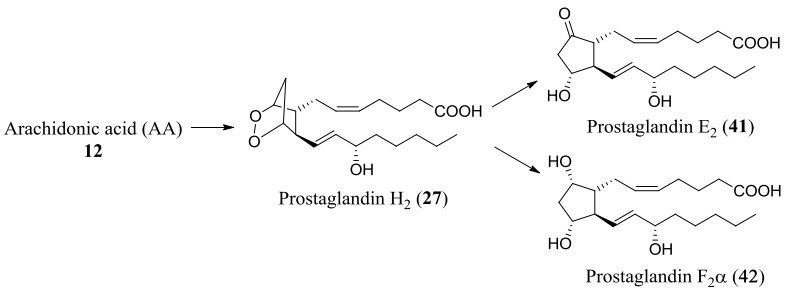


Figure 1.11. Biosynthetic pathway of $PGE_{2\alpha}$ and PG $F_{2\alpha}$.

The prostaglandins of the 3-series are derived from EPA (**7**) (Figure 1.12).

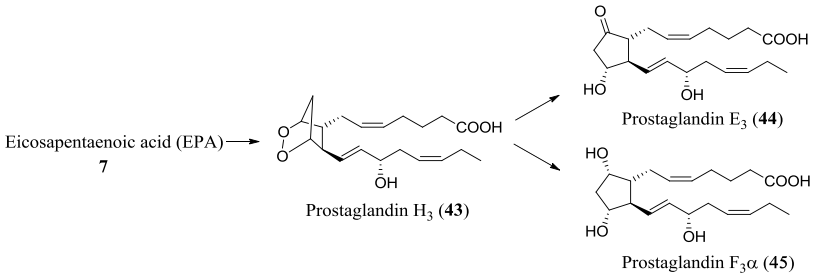


Figure 1.12. Biosynthetic pathway of $PGE_{3\alpha}$ and $PGF_{3\alpha}$.

1.7.1.2. Prostacyclin

Prostacyclin or prostaglandin I_2 (PGI_2, **46**) is produced in endothelial cells from prostaglandin H_2 (PGH_2, **27**) by the action of the enzyme prostacyclin synthase (Figure1.13).[99]

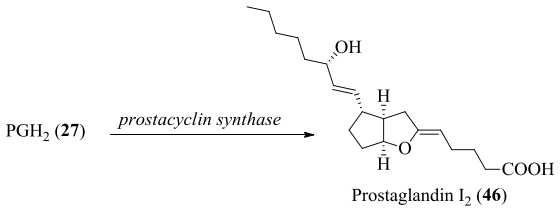


Figure 1.13. Biosynthetic pathway of PGI_2.

1.7.1.3. Thromboxanes

The two main thromoboxanes are thromboxane A_2 (TXA_2, **47**) and thromboxane B_2 (TXB_2, **48**), which are characterized by the presences of a 6-membered cyclic ether (Figure 1.14).[100]

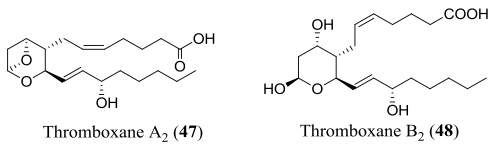


Figure 1.14. Biosynthetic pathway of TXA_2 and TXB_2.

1.7.1.4. Leukotrienes

Samuelsson and co-workers discovered the leukotrienes in white cells derived from bone marrow, *i.e.* leukocytes. These compounds have three double bonds in conjugation. The leukotrienes are synthesized in the cell from arachidonic acid (**12**) by 5-lipoxygenase (Figure 1.15).[80-82]

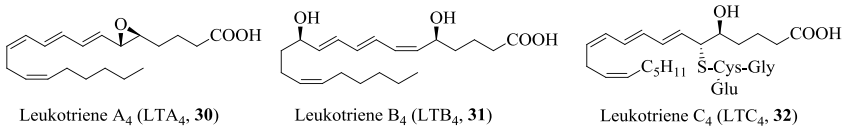


Figure 1.15. Examples of leukotrienes.

1.7.2. Polyunsaturated natural products

Bacillariolide I and its isomer bacillariolide II are unique eicosanoids which have been isolated from the wild and cultured cells of the diatom, *Pseudo-nitzschia multiseries.*[101] They possess a γ-lactone fused cyclopentanol framework with four stereocenters in addition to four methylene-interrupted double bonds (Figure 1.16). Bacillariolide I was reported as an inhibitor of phospholipase A_2 (PLA_2).[102]

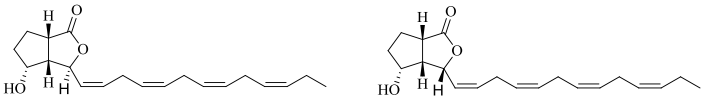


Figure 1.16. The structure of bacillariolides I and II.

The biosynthesis of bacillariolides seems to be closely related to the leukotriene biosynthesis and can be explained by the initial formation of 5(*S*)-hydropyroxy eicosapentaenoic acid (5-HPEPE, **51**) from EPA (**7**) which rearranged to the hydroxyl peroxide (**52**). The final step will then be the carbon ring closure by anionic opening of the epoxide (Figure 1.17).[101]

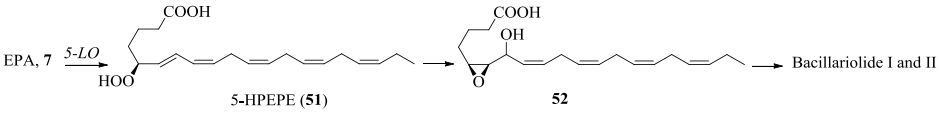


Figure 1.17. Biosynthetic pathway of bacillariolides I,II.

Salvà and co-workers isolated fourteen pyrrole-based metabolites coined mycalazol and mycalazal from the sponge *Mycale micracanthoxea*.[103] These natural products contain long carbon chain attached to the pyrrole ring at position-5. Some of them contain several methylene-interrupted *cis*-double bonds, *i.e.*: mycalazol 1 (**53**) and 2 (**54**) contain the same number of double bonds as in DHA (**9**) and as in α-linolenic acid (**3**), respectively. Mycalazol 5 (**55**) and mycalazal 2 (**56**) contain the same number of double bonds as in EPA (**7**) (Figure 1.18).

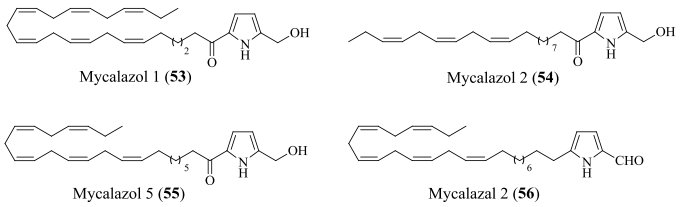
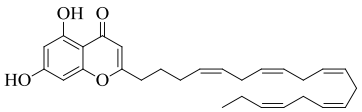


Figure 1.18. Pyrrole-based metabolites.

Platteli and Tringali reported the isolation and structure elucidation of *all*-(*Z*)-5,7-dihydroxy-2-(4*Z*,7*Z*,10*Z*,13*Z*,16*Z*-nonadecapentaenyl) chromone (**57**) from the pacific brown algae *Zonaria tournefortii*.[104] This polyunsaturated chromone metabolite possess the same number of methylene *Z*-interrupted double bonds as in EPA (**7**) (Figure 1.19).



all-(*Z*)-5,7-dihydroxy-2-(4*Z*, 7*Z*, 10*Z*, 13*Z*, 16*Z*-nonadecapentaenyl) chromone (**57**)

Figure 1.19. Chromone-based metabolite.

1.8. Stereoselective synthesis of *cis*-double bonds

Polyyne-semihydrogenation and Wittig reactions are considered the most two common strategies to synthesize polyunsaturated fatty acids and their metabolites.[3]

For the polyyne-semihydrogenation sequence, numerous systems have been reported for the reduction of a triple bond to a (*Z*)-double bond. In 1952, Lindlar reported that hydrogenation of alkynes under palladium catalysis (palladium oxide deposited on $CaCO_3$ or $BaSO_4$, and poisoned with lead acetate and quinoline) led to (*Z*)-alkenes with high selectivity.[105] Brown published another selective catalyst in 1973 which is known as the P-2 Ni catalyst. P2-Ni is generated *in situ* by reduction of nickel acetate with sodium borohydride in ethanol.[106] Many other systems have later been developed for the semi-hydrogenation of alkynes, such as triphenyl-phosphine copper hydride developed by Stryker and co-workers,[107] and tantalum chloride or titanium *tetra*-isopropoxide isopropyl magnesium chloride system.[108]

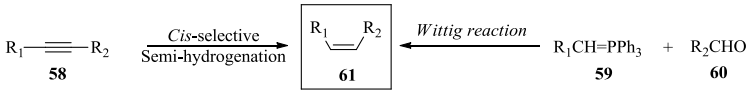


Figure 1.20. The routes for the synthesis of *cis*-alkenes.

For the Wittig reaction, the product may be formed as a mixture of *cis*- and *trans*-isomers, but each of these can be improved by appropriate reaction condition.[109] The *cis*-isomer is the dominate product when the reaction is carried out at low temperature (-100 ^{o}C), at high dilution and absence of lithium. Sodium *bis*-(trimethylsilyl) amide [$NaN(SiMe_3)_2$] is recommended as base.[3]

All of the aforementioned described methods have been used for the construction of (*Z*, *Z*)-1,4-dienes which are useful for the synthesis of methylene interrupted PUFAs. However, these methods can lead to mixtures of *cis*- and *trans*-isomers in the case of conjugated systems or highly unsaturated substrates.[110] In 1987, Boland and co-workers developed a method to activate zinc by mixing it with silver and copper to produce a Zn(Cu/Ag) system.[111] This system is efficient for the reduction of the conjugated alkynes to afford the corresponding *Z*-alkenes (Figure 1.21), but this system showed some limitation in the case of sensitive compounds or highly unsaturated compounds.[112-114]

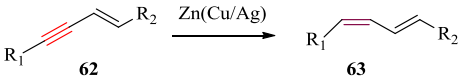


Figure 1.21. Selective semi-hydrogenation of conjugated alkynes by the Zn(Cu/Ag) system.

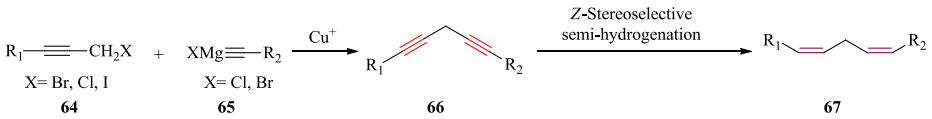
1.9. Synthesis of polyunsaturated fatty acids

It is well established that PUFAs have an important role in physiology and biochemistry. They are precursors for the biosynthesis of eicosanoids and hormone-like signaling molecule. However, animal lipids are not practical as starting materials for the preparation of large quantities of pure PUFAs required for comprehensive biological and nutritional investigations. Hence, synthetic studies are still desirable for this purpose. Herein is the different strategies used for the synthesis of PUFAs outlined.

1.9.1.Synthesis of PUFAs *via* acetylenic intermediate

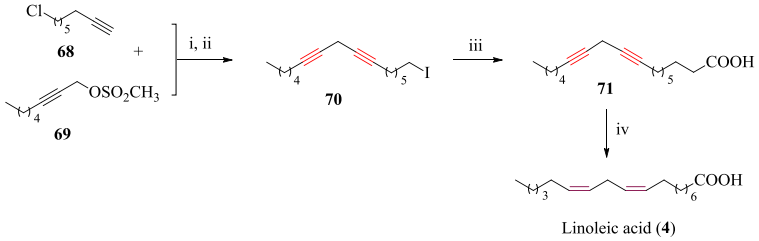
The synthesis of the (*Z*,*Z*)-1,4-diene system is initialized by the formation of a skipped diyne by reacting a propargyl halide derivative with an alkynyl magensium halide followed by a *Z*-stereoselective reduction to the diyne system.[3]

The method can be generalized by the following Scheme 1.2.



Scheme 1.2. Construction of the (*Z*,*Z*)-1,4-diene system.

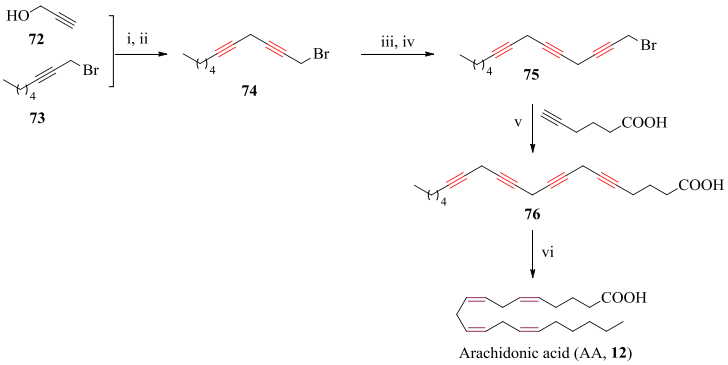
Linoleic acid (**4**) was the first PUFA that was synthesized by this approach by Rafael and Sondheimer in 1950 (Scheme 1.3).[115]



i) EtMgBr, Et_2O; ii) NaI, acetone; ii) $NaCH(CO_2Et)_2$; iv) H_2, $Pd(CO_3)_2$, EtOAc.

Scheme 1.3. The first total synthesis of linoleic acid (**4**).

In 1959, Osbond and co-workers synthesized arachidonic acid (AA, **12**) based on iterative use of propargyl alcohol. This synthetic protocol is considered as a general route to synthesize methylene interrupted PUFA (Scheme 1.4).[116] Docosahexaenoic acid (**9**) was also prepared by a similar stratgy.[117]



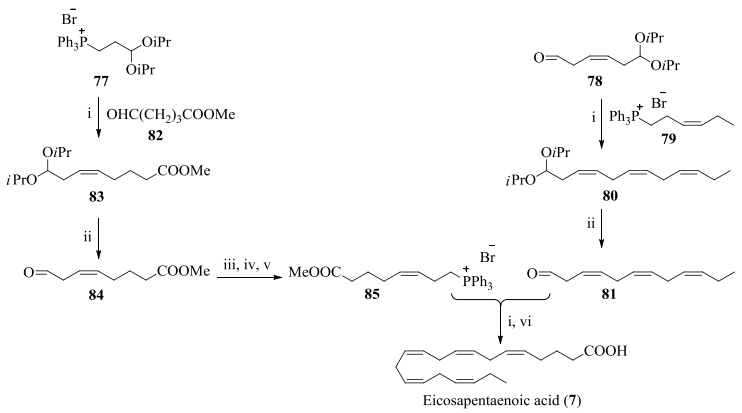
i) EtMgBr, CuCl; ii) PBr_3; iii) **72,** EtMgBr, CuCl; iv) PBr_3; v) EtMgBr, CuCl; vi) H_2/Pd-Lindlar.

Scheme1.4. The first total synthesis of arachidonic acid (**12**).

1.9.2. Synthesis of PUFAs using the Wittig reaction

The Wittig reaction is a reaction between a carbonyl compound and a phosphonium halide salt. The product can very often be a mixture of *cis*- and *trans*-isomers, but each of these can be optimized by appropriate reaction condition.[109]

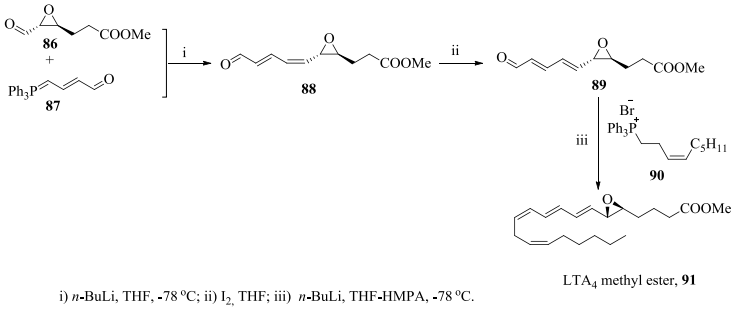
Eicosapentaenoic acid (EPA, **7**) was synthesized by employing the C-3 homologating agent **77**.[118] The synthesis of EPA started with the first Wittig reaction between C-3 homologating agent **77** with aldehyde **82** to afford compound **84** that was reduced by sodium borohydride followed by bromination and phosphorylation affording the phoshphonium salt **85**. *All*-(*Z*)-trienic aldehyde **81** was synthesized by a Wittig reaction between aldehyde **78** with phosphonium salt **79**, affording compound **80** that was hydrolyzed under acidic conditions to yield aldehyde **81**. This aldehyde was reacted with phosphonium salt **85** yielding EPA (**7**) with the correct all *Z*-stereochemical geometry (Scheme 1.5).



i) $NaN(SiMe)_2$, THF, -90 °C; ii) THF, camphorsulfonic acid; iii) $NaBH_4$, EtOH; vi) CBr_4, Ph_3P, CH_2Cl_2; v) Ph_3P, acetonitrile, Δ; vi) LiOH, THF/H_2O.

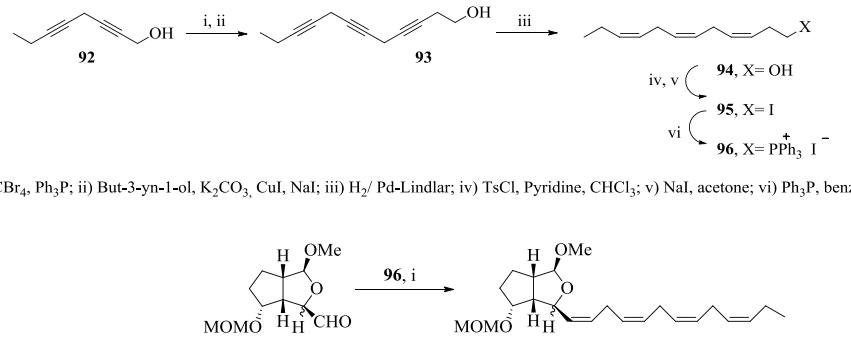
Scheme 1.5. Synthesis of EPA.

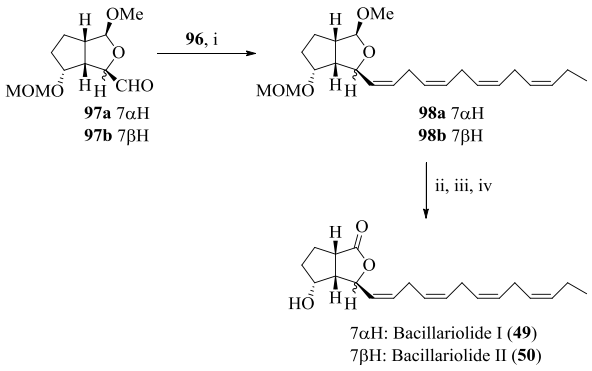
Another example of the use of the Wittig reaction is the synthesis of leukotriene A_4 methyl ester **91**.[119] The first step of this protocol was the reaction of the conjugated aldehyde **86** with the stabilized ylide **87** to afford mainly the *cis*-isomer **88** unexpectedly. The second Wittig reaction between the non-stabilized Wittig reagent **90** with aldehyde **89** gave the expected *cis*-isomer **91**. It is noted that in this synthetic approach, the epoxide and ester functional groups were not affected during the two Wittig reactions in this synthesis (Scheme 1.6).



Scheme 1.6. Synthesis of leukotriene A_4 methyl ester.

A combination of alkyne synthesis and the Wittig reaction was employed in the synthesis of bacillariolide I and II.[120] The skipped triene-phosphonium iodide **96** was synthesized by the polyyne-semihydrogenation sequence. Coupling reaction of aldehyde **97a** or **79b** with Wittig reagent **96** afforded *all-Z*-tetraene **98**. Hydrolysis of methyl acetal **98a** or **98b** by treatment with aqueous acetic acid, oxidation of hemiacetal with Jones reagent followed by removal of methoxymethyl group with acetic acid and concentrated hydrochloric acid afforded bacillariolide I (**49**) and bacillariolide II (**50**) (Scheme 1.7).

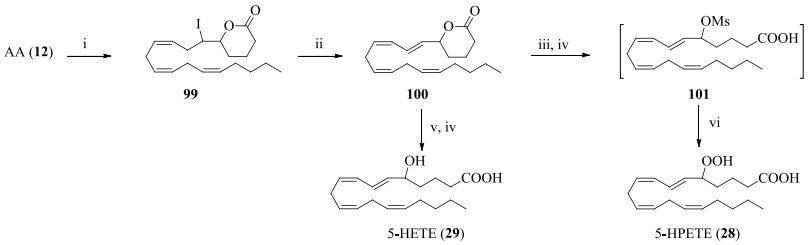




Scheme 1.7. Synthesis of bacillariolide I and II.

1.9.3. Hemisynthseis of PUFAs

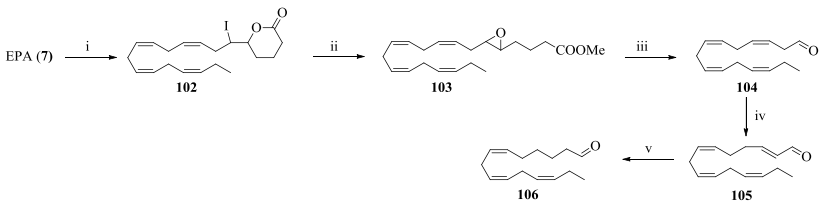
Corey and co-workers have reported an efficient protocol for the synthesis of many eicosanoids using arachidonic acid as starting material.[121-123] Iodolactonazition of arachidonic acid (**12**) afforded the iodolactone **99** which was treated with DBU to give the corresponding teteraene lactone **100**, a precursor to 5-HPETE (**28**) and 5-HETE (**29**) (Scheme 1.8).[124]



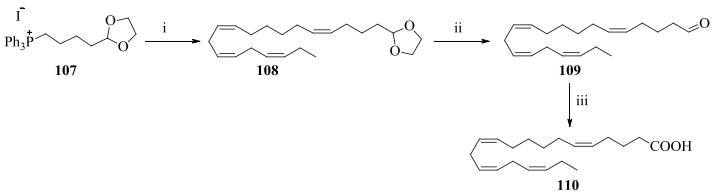
i) $KHCO_3$, KI, I_2, THF, H_2O; ii) DBU, benzene; iii) CH_3SO_2Cl, Et_3N, CH_2Cl_2; iv) LiOH, DME, H_2O; v) Et_3N, MeOH; vi) H_2O_2, Et_2O.

Scheme 1.8. Synthesis of 5-HPETE and 5-HETE.

Juniperonic acid (**110**) was synthesized using the C-15 aldehyde **104** as the key intermediate that was formed *via* the iodolactonization of EPA (**7**). The Wittig reaction between aldehyde **106** and triphenyl phosphonium iodide **107** afforded the acetal **108** that was deprotected to give the aldehyde **109**. This aldehyde was oxidized by a Pinnick oxidation to afford juniperonic acid (**110**) (Scheme 1.9).[125]



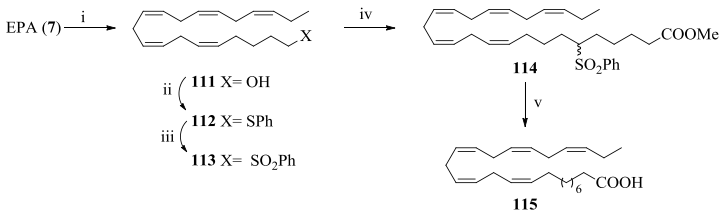
i) I_2, $KHCO_3$, KI, THF, H_2O; ii) K_2CO_3, MeOH; iii) H_5IO_6, Et_2O; iv) DBU, Et_2O; v) DIBAl-H, CuI, THF-HMPA.



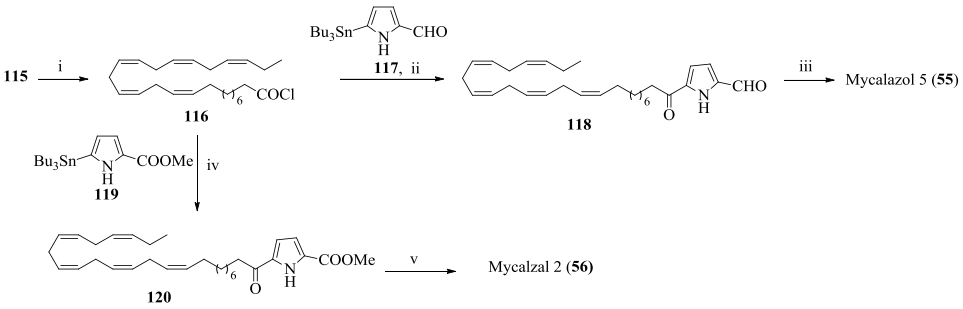
i) **106**, NaHMDS, -100 °C, THF; ii) HCOOH (aq), 1,4-dioxane, 80 °C; iii) 80% $NaClO_4$, NaH_2PO_4, 2-methyl-2-butene, $MeCN/H_2O$.

Scheme 1.9. Synthesis of juniperonic acid (**110**).

In 2004, Skattebøl and Hansen described the synthesis of two polyunsaturated pyrroles, mycalazol 5 (**55**) and mycalazal 2 (**56**) starting from EPA (**7**) (Scheme 1.10).[126]



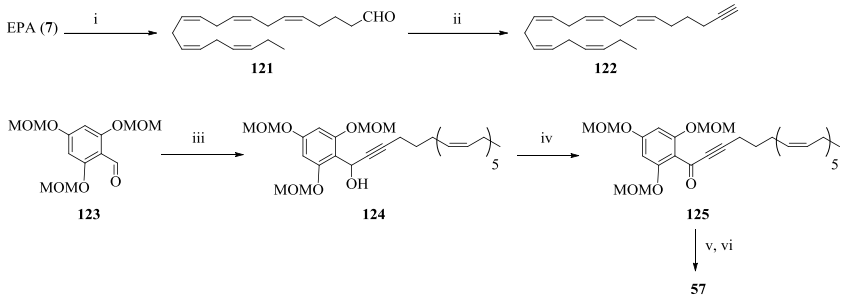
i) $LiAlH_4$, Et_2O, Δ; ii) $(PhS)_2$, Bu_3P, THF; iii) $KHSO_5$, MeOH, 0 °C; iv) (a) LDA, THF, -78 °C, (b) Methyl 5-bromovalerate, THF; v) (a) Na(Hg), Na_2HPO_4, MeOH, -20 °C, (b) LiOH, H_2O, MeOH.



i) $(COCl)_2$, CH_2Cl_2; ii) $Pd(PPh_3)_4$, THF, Δ; iii) $Zn(BH_4)_2$, THF, 0-25 °C; iv) (a) NaCN, CH_2Cl_2, (b) MnO_2, MeOH; v) $Pd(PPh_3)_4$, THF, Δ; vi) $TsNHNH_2$, MeOH, (b) $NaB(CN)H_3$, MeOH; vii) DIBAl-H, -78 °C, THF.

Scheme 1.10. Synthesis of mycalzol 5 and mycalazal 2.

Another synthetic protocol was investigated by Anwar and Hansen again using EPA (**7**) as starting material for the synthesis of *all*-(*Z*)-5,7-dihydroxy-2-(4*Z*,7*Z*,10*Z*,13*Z*,16*Z*-nonadecapentaenyl)chromone (**57**) (Scheme 1.11).[127]

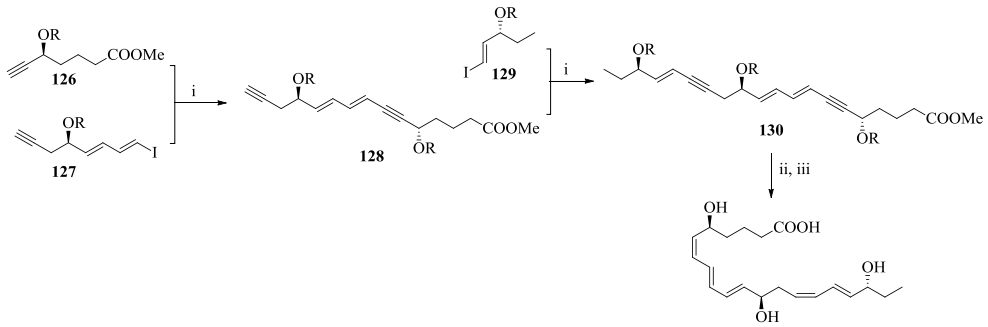


i) DIBAl-H, Toluene, -78 °C; ii) LDA, $TMSCHN_2$, THF, -78 °C; iii) **122**, *n*-BuLi, THF, -78 °C, iv) MnO_2, CH_2Cl_2, v) HCl, EtOH; vi) K_2CO_3, acetone, 60 °C

Scheme 1.11. Synthesis of polyunsaturated chromone metabolite.

1.9.4. Synthesis of PUFAs by cross coupling reactions

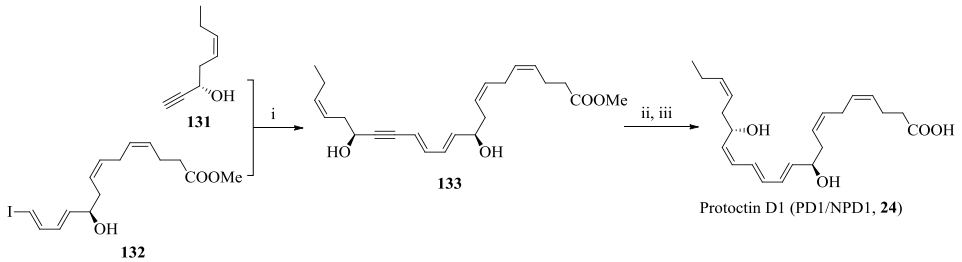
The first total synthesis of resolvin E1 was reported by Serhan and Petasis and their co-workers by using Pd-catalyzed cross coupling reactions of the building blocks **126** and **127** to form **128**. This terminal alkyne was coupled with vinylic iodide **129** to afford the *bis*-acetylenic precursor **130** that was reduced *via* stereocontrolled reduction to RvE1 (**22**) (Scheme 1.12).[128]



i) $Pd(PPh_3)_4$, CuI, benzene, rt; ii) Zn(Cu/Ag), $MeOH/H_2O$, 40 °C; iii) NaOH, $MeOH/H_2O$, rt.

Scheme 1.12. Synthesis of resolvin E1 (RvE1).

Protectin D1/Neuroprotectin D1 (PD1/NPD1) was also synthesized *via* Pd-catalyzed coupling reaction of the two building blocks **131** and **132** to afford the acetylenic precursor **133** that was converted to PD1/NPD1 (**24**) *via* *Z*-selective semi-reduction and hydrolysis (Scheme 1.13).[129]

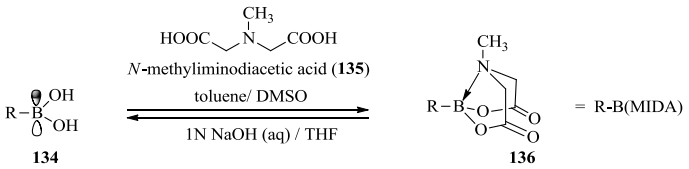


i) $Pd(PPh_3)_4$, CuI, benzene, rt; ii) Zn(Cu/Ag), $MeOH/H_2O$, 40 °C; iii) NaOH, $MeOH/H_2O$, rt.

Scheme 1.13. Synthesis of protectin D1/neuroprotectin D1 (PD1/NPD1).

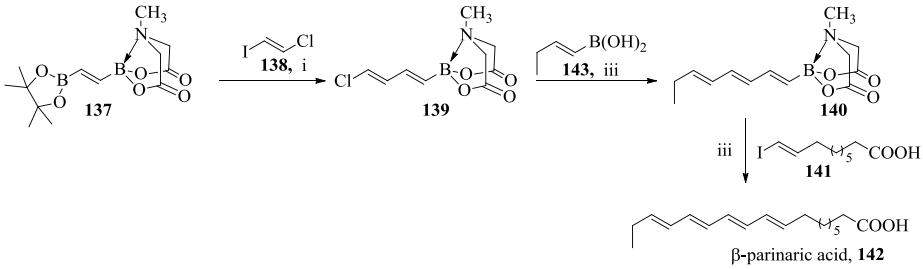
1.10. Syntheses of polyene system *via* iterative cross coupling (ICC) strategy

An iterative synthetic approach is a powerful strategy that facilitate the synthesis of biopolymers, *i.e.* polypeptides, oligonucleotide and oligosaccharides.[130-132] Recently, this strategy was employed in the synthesis of natural products.[128,133,134] Burke and co-workers discovered that the trivalent *N*-methyliminodiacetic acid (MIDA) ligand (**135**) deactivates and blocks the boron center **134** by rehybridization to sp^3configured **136**.[135] Consequently, the resulting MIDA boronates **136** are inactive towards transmetallation under anhydrous cross-coupling conditions. They are tolerant of the Heck, Stille, Suzuki–Miyaura, and Sonogashira coupling protocols.[135,136]



Scheme 1.14. Formation of sp^3 hybridized boron.

Burke and co-workers have employed the MIDA boronates in modular syntheses of some polyene natural products.[133-136] The conjugated tetraene polyunsaturated fatty acid, β-parinaric acid **142** was synthesized in three steps. The initial step is the Suzuki-Mayura reaction between the MIDA boronate **137** with the (*E*)-1-chloro-2-iodoethene **138** to form the *di*-functional dienyl chloride MIDA boronate **139** that was coupled with (*E*)-1-butenylboronic acid to afford the *all-trans* trienyl boronate **140**. The boron deprotection of **140** was achieved under mild aqueous basic conditions, and subsequent cross-coupling with vinyl iodide **141** afforded β-parinaric acid (**142**).[134]



i) $PdCl_2(dppf)$, K_3PO_4, DMSO; ii) Pd(OAc), X-Phos, K_3PO_4; iii) Pd(OAc), X-Phos, 1N NaOH.

Scheme 1.15. Synthesis of β-parinaric acid (**142**).

Burke and co-workers developed haloalkenyl MIDA boronates **143-146** in all possible stereomeric form that enable the synthesis of different polyene systems (Figure 1.20).[137]

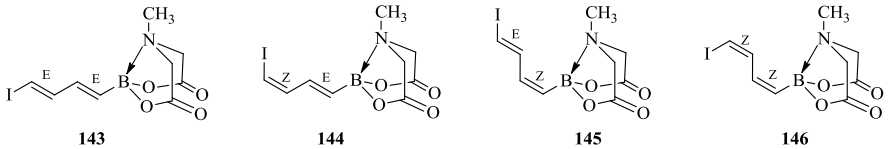
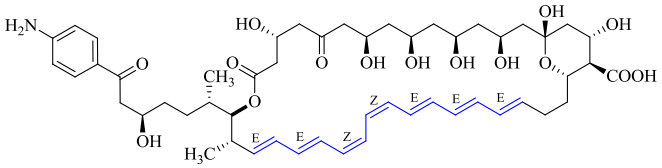
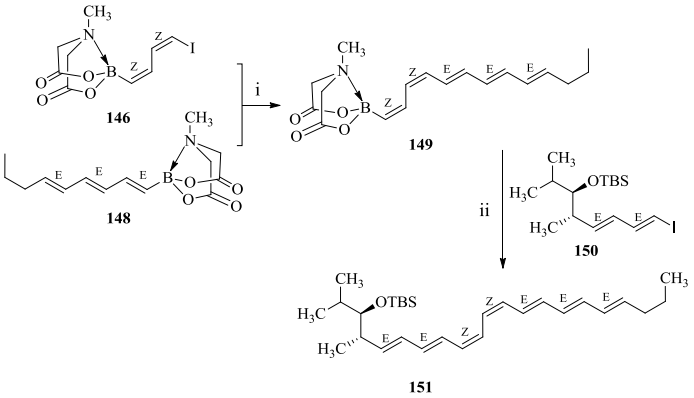


Figure1.22. Haloalkenyl Mida boronate **144-146**.

As an application for the utility of these MIDA boronates was the synthesis of the highly complex (*E*,*E*,*E*,*Z*,*Z*,*E*,*E*)-heptaene moiety found in vacidin A (**147**) (Scheme. 1.16).[137]



Vacidin A (**147**)
Polyketide



i) $Pd(OAc)_2$, X-Phos, Cs_2CO_3, THF, 35 ^oC; ii) $Pd(OAc)_2$, SPhos, 1N aq. NaOH, THF, 23 ^oC.

Scheme 1.16. Synthesis of (*E*,*E*,*E*,*Z*,*Z*,*E*,*E*)-heptaene moiety.

1.11. Aim of Study

- In this study, the aim was to use eicosapentaenoic acid (EPA) and eicosanoic acid (EA) as starting materials for the development of efficient synthesis of polyunsaturated pyrrole and polyunsaturated chromone derivatives. The newly synthesized analogs were subjected to biological testing.

- In this thesis, the aim was also to synthesize bosseopentaenoic acid *via* an iterative cross coupling strategy using MIDA boronates.

- Another study aimed at investigating the development of a reduction procedure for conjugated alkynes and non-activated alkynes to *Z*-alkenes by modification of the Zn(Cu/Ag) system initially investigated by Boland and co-workers.

- The thesis also describes an attempted synthesis of α-parinaric acid.

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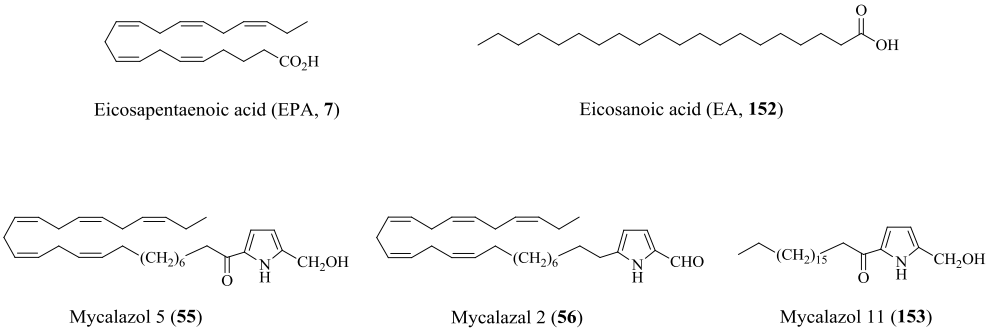
2. Results and Discussion

2.1. Paper (I): Synthesis of mycalazol and mycalazal analogs with potent antiproliferating activities

Due to the limited information on structure activity relationship of the pyrrole-based metabolites within the mycalazols and mycalazals class of metabolites,[1] we aimed to synthesize some analogs with saturated and unsaturated C-20 alkyl chain at the position-5 of the pyrrole ring. We decided to use acids **7** and **152** as starting materials. The cytotoxic activity and structure activity relationship of the newly synthesized pyrroles were investigated. We also decided to prepare 2-acyl and 2-alkyl substituted thiophene derivatives with an unsaturated and saturated C-20 side chain to compare these compounds with the pyrrole-containing analogs.



Eicosapentaenoic acid (EPA, **7**)

Eicosanoic acid (EA, **152**)

Mycalazol 5 (**55**)

Mycalazal 2 (**56**)

Mycalazol 11 (**153**)

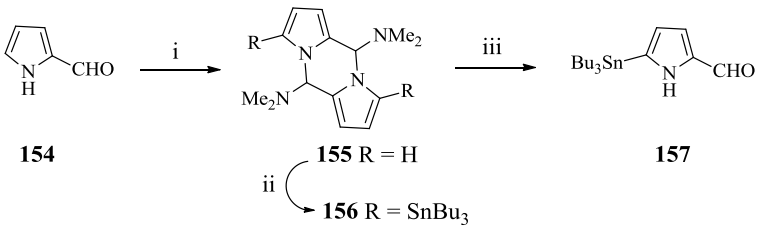
Figure 2.1. Structures of some polyunsaturated pyrrole-containing metabolites.

The structurally related 2,5-disbstituted pyrroles isolated from the northeastern Atlantic sponge *Mycale micracanthoxea*, have been reported to be cytotoxic agents against five different cancer cell lines. These natural products contain either a saturated or an unsaturated carbon chain attached to the 5-position.[2]

Abell and Nabb reported the synthesis and cytotoxicity of mycalazol 11 (**153**) and some related analogs.[1] Recently Skattbøl and Hansen reported the synthesis of the polyunsaturated pyrrole metabolites mycalazol 5 (**55**) and mycalazal 2 (**56**) starting from eicosapentaenoic acid (EPA, **7**).[3]

2.1.1. Synthesis

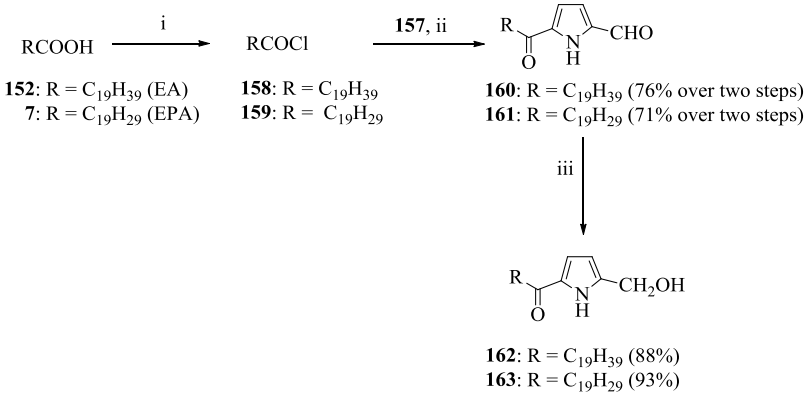
Our strategy for the synthesis of the mycalazol 5 (**55**) and mycalazal 2 (**56**) analogs was based on a Stille coupling[4] between the 5-(tri-*n*-butylstannyl)pyrrole-2-carboxaldehyde (**157**)[5] and the corresponding acid chlorides of eicosapentaenoic acid (EPA, **7**) and eicosanoic acid (EA, **152**). The synthesis of stannyl compound **157** started with the preparation of the dimer **155** from commercial available pyrrole-2-carboxaldehyde (**154**) and 40% aqueous dimethyl amine using a literature procedure[6] to afford compound **155** in quantitative yield. Lithiation of compound **155** was accomplished with 2.3 equivalents of *n*-butyllithium, and then reaction with tri-*n*-butylstannyl chloride provided compound **156**. Aqueous hydrolysis of compound **156** with sodium acetate afforded stannyl compound **157** in 57% overall yield from 2-pyrrole-carboxyaldehyde (**154**) (Scheme 2.1).



i) $HNMe_2$, H_2O, 25 °C ii) a) *n*-BuLi, THF b) *n*-Bu_3SnCl, THF, -78 - 25 °C
iii) 0.4 M NaOAc, H_2O, THF, Δ (57% from **154**).

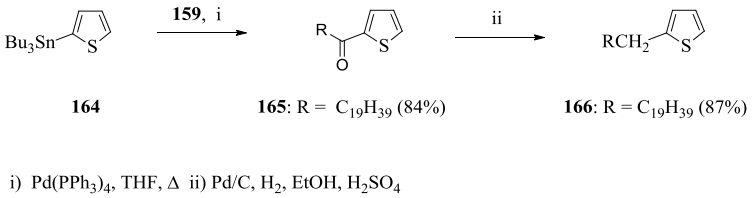
Scheme 2.1. Synthesis of 5-(tri-*n*-butylstannyl)pyrrole-2-carboxaldehyde **157**.

The synthesis of the acid chlorides **158** and **159** were carried out by treating EA (**152**) and EPA (**7**) with excess oxalyl chloride in dichloromethane. This afforded the acid chlorides **158** and **159** in quantitative yields. Subsequently, a Stille coupling of the acid chlorides with the stannylpyrrole carboxaldehyde **157** afforded the analogs **160** and **161** in 76% and 71% yield, respectively. The reduction of the formyl group was carried out selectively in the presence of the carbonyl group and the double bonds in the unsaturated chain (at postion-5) using freshly prepared $Zn(BH_4)_2$[7] in THF at 0-25 °C. The analogs **162** and **163** were obtained in 88% and 93% yields, respectively (Scheme 2.2).



Scheme 2.2. Synthesis of analogs **160-163**.

The synthesis of 2-acyl thiophene analog **165** was carried out using a Stille coupling between acid chloride **159** and commercially available 2-(*tri*-*n*-butylstannyl)pyrrole (**164**) to afford **165** in 84% yield. Reduction of the carbonyl group using Pd/C and H_2 in the presence of catalytic amounts of sulphuric acid provided compound **166** in 87% yield (Scheme 2.3).



Scheme 2.3. Synthesis of thiophene analogs **165** and **166**.

2.1.2. Biological studies

Mycalazol 5 (**55**) and mycalazal 2 (**56**), the synthesized pyrrole analogs **160-163** and the thiophene analogs **165** and **166** were tested against four human cancer cell lines; two types of ovarian cancer cell lines (SKOV and OVCAR) and two types of melanoma cancer cell lines (WM35 and WM239). These were compared with the original lines (VERO). All the compounds exhibited potent cytotoxic activity with IC_{50}-values in the nanomolar range, except compound **166**, which was inactive.

Mycalazol 5 (**55**) and mycalazal 2 (**56**) were more active than the prepared analogs.

Mycalazol 5 (**55**) was slightly more cytotoxic than mycalazal 2 (**56**). This result was in agreement with the previous report by Salvá and co-workers.[2] The two pyrrole-containing analogs **160** and **161** were the most active compounds among the other prepared analogs. It was observed that compound **161** (IC_{50}-values 11.3-22.9 nM) showed higher activity than compound **160 (**IC_{50}-values 15.9-31.1 nM). It was observed that the 2-acyl substituted thiophene **165** showed decrease cytotoxicity compared to the other analogs. The 2-alkyl thiophene **166** was inactive towards all four cancer cell lines and the VERO cells. All the other analogs have potent cytotoxic effect on the original cells (VERO). Hence, no selectivity was observed. The biological results are summarized are in Table 2.1.

Compounds	SKOV[a]	OVCAR[a]	WM35[a]	WM239[a]	VERO[a]
55	14.9 (±2.1)	9.4 (±1.8)	2.9 (±0.9)	2.1 (±0.9)	2.1 (±0.9)
56	23.3 (±3.0)	18.0 (±2.1)	4.1 (±1.0)	4.3 (±0.8)	4.3 (±0.8)
160	31.1 (±2.3)	24.1 (±1.5)	13.0 (±1.3)	15.9 (±1.9)	15.9 (±1.9)
161	22.9 (±1.9)	21.7 (±2.8)	11.3 (±1.9)	12.1 (±2.1)	12.1 (±2.1)
162	38.1 (±2.1)	38.5 (±2.2)	36.4 (±2.1)	27.7 (±2.2)	27.7 (±2.2)
163	34.3 (±3.3)	32.7 (±4.1)	24.1 (±2.6)	20.5 (±2.9)	20.5 (±2.9)
165	49.1 (±2.1)	58.7 (±3.3)	45.5 (±3.3)	58.0 (±2.9)	58.0 (±2.9)
166	>100	>100	>100	>100	>100

[a]Values (nM) are means of three experiments in each cell assay. Standard deviation is given in parentheses

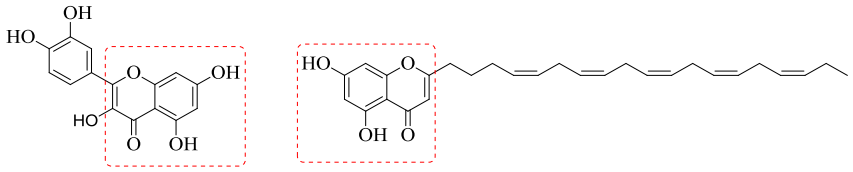
Table 2.1.

The results of this study have been described in **Paper I** and the experimental procedure and spectroscopic data are attached in the appendix.

2.2. Paper (II): Polyunsaturated fatty acid-derived chromones exhibiting potent antioxidant activity

The aim of this study was to develop efficient synthesis of some analogs of polyunsaturated chromone metabolite **57** and to investigate their susceptibility to oxidation.

Several diseases are associated with cellular oxidation processes. These oxidation processes may be retarded in the presence of antioxidants. Oxidative DNA damage is associated with cancer,[8,9] aging and neurodegenerative diseases such as Alzheimer's and Parkinson's[10-14] and cardiovascular diseases such as arteriosclerosis.[15] Therefore, prevention of oxidative stress caused by reactive oxygen/nitrogen species (ROS/RNS) has important implications in prevention and treatment of such diseases. Phenolic compounds, such as quercetin (**167**) are recognized for their antioxidant activity due to their ability to scavenge free radical. These compounds sacrificially reduce the ROS/RNS preventing damage to biomolecules or formation of more ROS.[16] However, limited bioavailability of quercetin (**167**) has been reported.[17] It has been reported that EPA (**7**) showed potent antioxidant effect by reducing the formation of ROS.[18] From this practical point of view and in continuation of the previous work on the synthesis and biological evaluation of polyunsaturated natural products,[19] we became interested in using the chromone-based metabolite **57** as a lead compound for preparing new lipophilic antioxidants. This natural product is characterized by possessing the same (5,7-dihydroxy chromone) moiety as that present in quercetin (**167**). Compound **57** also has an unsaturated chain containing five methylene-interrupted *cis*-double bonds as those present in EPA (**7**). The natural product **57** was isolated from different algae.[20] The total synthesis of **57** was reported using EPA (**7**) as starting material.[21] To the best of our knowledge, no biological data for this polyunsaturated natural product has been reported.



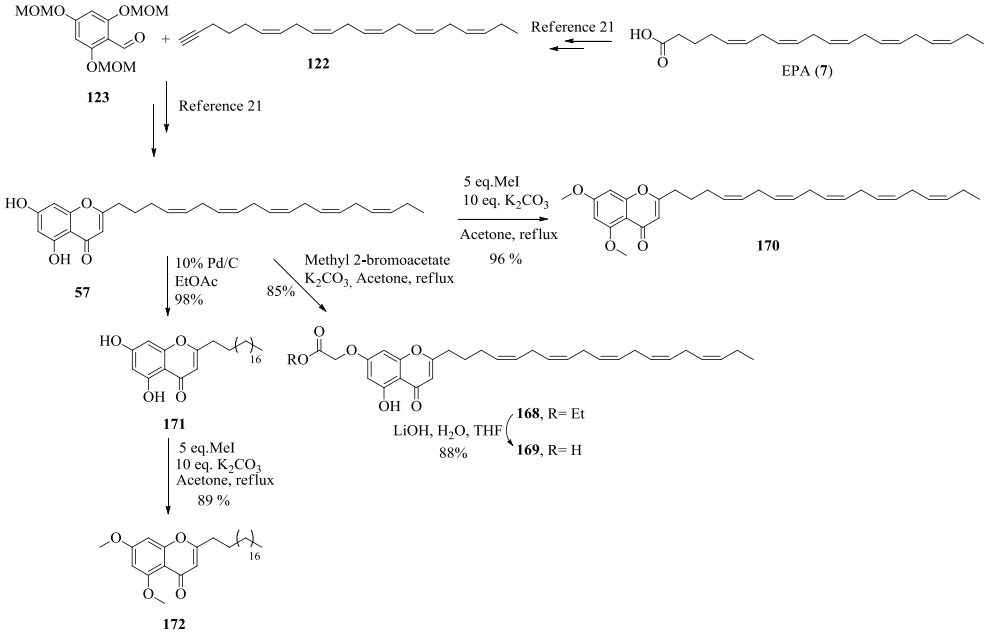
Quercetin (**167**)

all-(*Z*)-5,7-dihydroxy-2-(4*Z*, 7*Z*, 10*Z*, 13*Z*, 16*Z*-nonadecapentaenyl) chromone (**57**)

Figure 2.2. The structure of quercetin (**167**) and the natural product **57**.

2.2.1. Synthesis

Compound **57** was prepared as previously reported.[21] The hydroxyl group in compound **57** was alkylated to provide compound **168** in 85% yield. This compound was hydrolyzed under mild basic condition to afford acid **169** in 88% yield. Methylation of the hydroxyl groups in compound **57** provided compound **170**. Reduction of compound **57** using Pd/C (5 mol%) gave the saturated chain derivative **171** in 98% yield. Subsequently, this compound was methylated to afford compound **172** in 89% yield (Scheme 2.4).



Scheme 2.4. Synthesis of chromone analogs.

2.2.2. Synthesis of 3-(1-alkynyl) chromone derivatives

In this study, we developed new derivatives of the natural product **57** with polyunsaturated and saturated side chains attached to the position-3 in the chromone moiety. These analogs contain a triple bond in conjugation with the chromone ring.

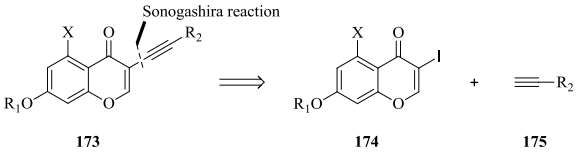
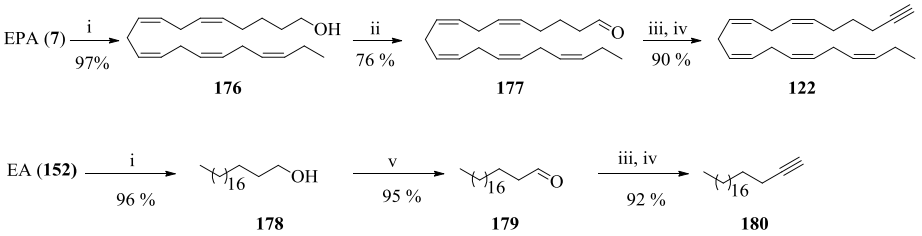


Figure 2.3. New chromone derivatives based on the Sonogashira reaction.

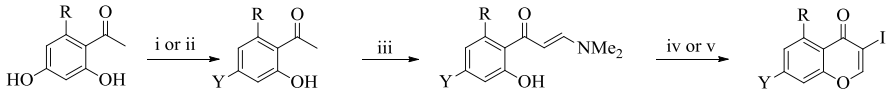
We started our synthesis of the chromone derivatives by preparing the alkynes **122**[21,22] and **180** by the Corey-Fuchs reaction[23] on the aldehydes derived from EPA (**7**) and EA (**152**) (Scheme 2.5).



i) $LiAlH_4$, Et_2O, 0 °C, 1h; ii) $(COCl)_2$, Et_3N, DMSO, -78 °C, 10 min;
iii) Zn, Ph_3P, CBr_4 , CH_2Cl_2 ; iv) 1.6 M *n*-BuLi, THF, -78 °C, 1h ; v) SiO_2, PCC, CH_2Cl_2 , 3h.

Scheme 2.5. Synthesis of the alkynes **122** and **180**.

We prepared two types of iodochromones using a literature procedure.[24,25] The synthesis of compound **184a** or **184b** started by the formation of the protected hydroxyl compounds **182a** or **182b**. These compounds were treated with dimethylformamide-dimethylacetal to afford enaminone **183a** or **183b**, respectively. The enaminone **183a** was reacted with iodine in dichloromethane in the presence of pyridine, which gave **184a** in 42% overall yield from **181a**, while the enaminone **183b** was reacted with iodine in methanol to afford compound **184b** in 39% overall yield from **181b** (Scheme 2.6).

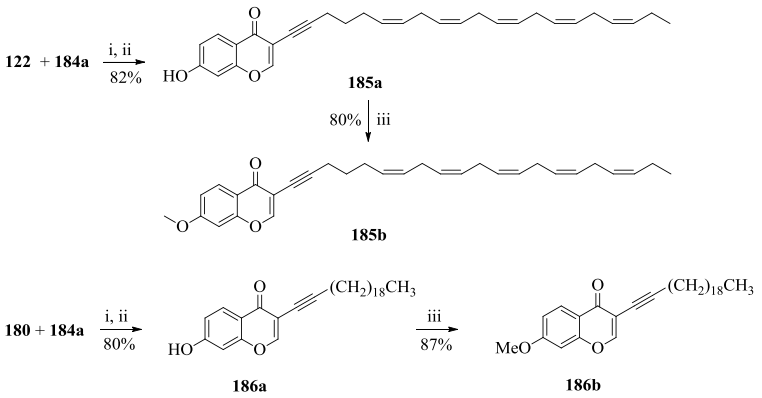


181a, R= H
181b, R= OH
182a, R= H, Y= OTHP
182b, R= OMOM, Y= OMOM, 54%
183a, R= H, Y= OTHP
183b, R= OMOM, Y= OMOM, 87%
184a, R= H, Y= OTHP, 42% from **181a**
184b, R= OMOM, Y= OMOM, 84%

i) DHP, PTSA, CH_2Cl_2; ii) MOMBr, K_2CO_3, Acetone, Δ, 1h; iii) DMFDMA, 95 °C, 1.5h;
iv) I_2, pyridine, CH_2Cl_2, rt, 24 h; v) I_2, MeOH, rt, 24h.

Scheme 2.6. Synthesis of iodochromones **184a**,**b**.

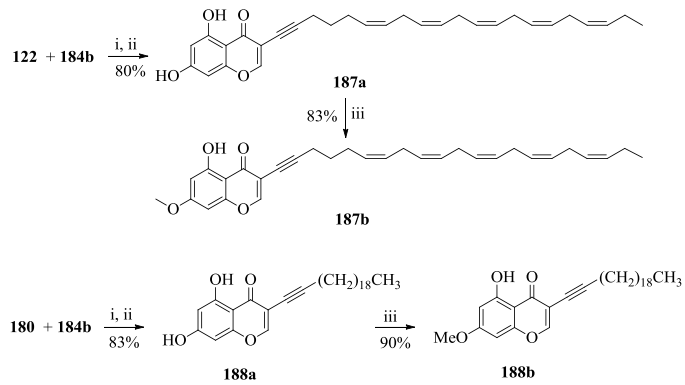
Alkyne **122** or **180** was reacted with iodochromone **184a** in a Sonogashira reaction.[26] The resulting products were reacted with *p*-toluenesulphonic acid (10 mol%) to afford chromone derivatives **185a** and **186a** in 82% and 80% yields, respectively. The hydroxyl groups in **185a** and **186a** were alkylated using MeI in acetone in the presence of K_2CO_3 to afford the analogs **185b** and **186b** in 80% and 87% yields, respectively.



i) $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, THF, 2 h; ii) PTSA, MeOH/THF, 60 °C, 1h ; iii) MeI, K_2CO_3, Acetone, Δ, 2h.

Scheme 2.7. Synthesis of the chromone analogs **185a**,**b** and **186a**,**b**.

Alkyne **122** or **180** was then reacted with iodochromone **184b**. The resulting products were refluxed in $MeOH/CH_3Cl$ in the presence of concentrated HCl to remove the methoxymethyl groups to afford compounds **187a** and **188a** in 80% and 83% yields, respectively. After the alkylation of **187a** and **188a**, compounds **187b** and **188b** were obtained in 83% and 93% yields, respectively (Scheme 2.8).



i) $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, THF, 2 h; ii) 1 ml conc. HCl, $MeOH/CH_3Cl$, Δ, 1 h ; iii) MeI, K_2CO_3, acetone, Δ, 2h.

Scheme 2.8. Synthesis of chromone analogs **187a,b** and **188a,b**.

2.2.3. Biological studies

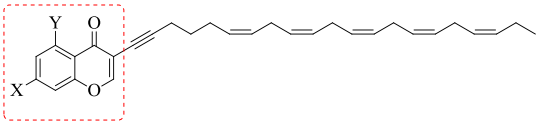
Compounds **57**, **167** and **169-172** were evaluated in the cellular lipid peroxidation antioxidant activity (CLPAA) assay using HepG2 cells.[27] The most potent compounds were quercetin (**167**) and the natural product **57** with IC_{50}= 10±3 and 14±9 μM, respectively. The analog **169** showed modest activity. Neither the dimethylated compounds **170** and **172**, nor compound **171** showed any inhibitory activity. From this point of view, it is observed that the compounds that contain free hydroxyl group with the polyunsaturated side chain have the potential to oxidize and prevent formation of ROS (Table 2.2).

Compound	IC_{50}[a] (μM)	IC_{50}[b] (μM)	ClogP[d]
57	14±9	160±25	8.4
167	10±3	71±39	1.5
169	29±3	342±122	8.9
170	> 1000	n.d.[c]	n.d.[c]
171	> 1000	n.d.[c]	n.d.[c]
172	> 1000	n.d.[c]	n.d.[c]

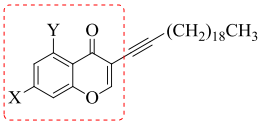
[a]:The IC_{50}-value was determined based on three experiments in the CLPAA assay
[b]: The IC_{50}-value was determined based on three experiments in the CAA assay
[c]: not determined
[d]: ClogP determined from the SciFinder Scholar database

Table 2.2.

The compounds **185a,b**, **186a,b**, **187a,b** and **188a,b** will also be subjected to a lipid peroxidation antioxidants and cytotoxic assay. The results from biological testing will be reported in due course (Figure 2.4.).



185a, X= OH, Y= H
185b, X= OCH_3, Y= H
187a, X= OH, Y= OH
187b, X= OCH_3, Y= OH



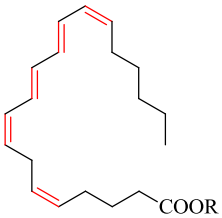
186a, X= OH, Y= H
186b, X= OCH_3, Y= H
188a, X= OH, Y= OH
188b, X= OCH_3, Y= OH

Figure 2.4.

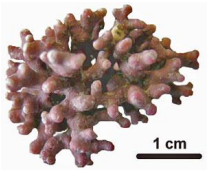
The results of this study have been described in **Paper II**, and the experimental procedure and spectroscopic data are attached in the appendix.

2.3. Paper (III): First total synthesis of methyl (5*Z*,8*Z*,10*E*,12*E*,14*Z*)-eicosapentaenoate

The aim of this study was to develop an efficient and stereoselective synthesis for bosseopentaenoic acid (**20**) or its methyl ester **189.**



20 (Bosseopentaenoic acid R= H)
189 (Bosseopentaenoic acid methyl ester R= Me)



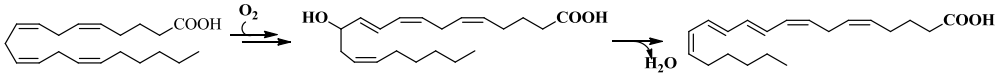
Phytochemistry **1993**, *34*, 1029–1033

Lipids **1995**, *30,* 583–589.

Figure 2.5. Bosseopentaenoic acid (**20**) and its methyl ester **189**.

Marine organisms have been shown to be a source for PUFAs and their related metabolites.[28] Many of these metabolites have interesting biological activity.[29] Bosseopentaenoic acid (**20**) was isolated from the red algae *Bossiella orbigniana* by Burgess and co-workers in 1991.[30] Subsequently, the methyl ester **189** was isolated by Gerwick and co-workers in 1993 from the red marine alga *Lithothamnion corallioides*, collected from the south coast of Norway.[31] In 1995, Jacobs and co-workers isolated the same ester along with other conjugated PUFAs from the green alga *Anadyomene stellata*.[32] This PUFA contains four conjugated double bonds with the (*Z*,*E*,*E*,*Z*)-moiety, along with one methylene interrupted *Z*-double bond; five double bonds in total.

The biosynthetic pathway for bosseopentaenoic acid (**20**) from arachidonic acid (AA, **12**) was proposed by Burgess and co-workers (Scheme 2.9).[30]

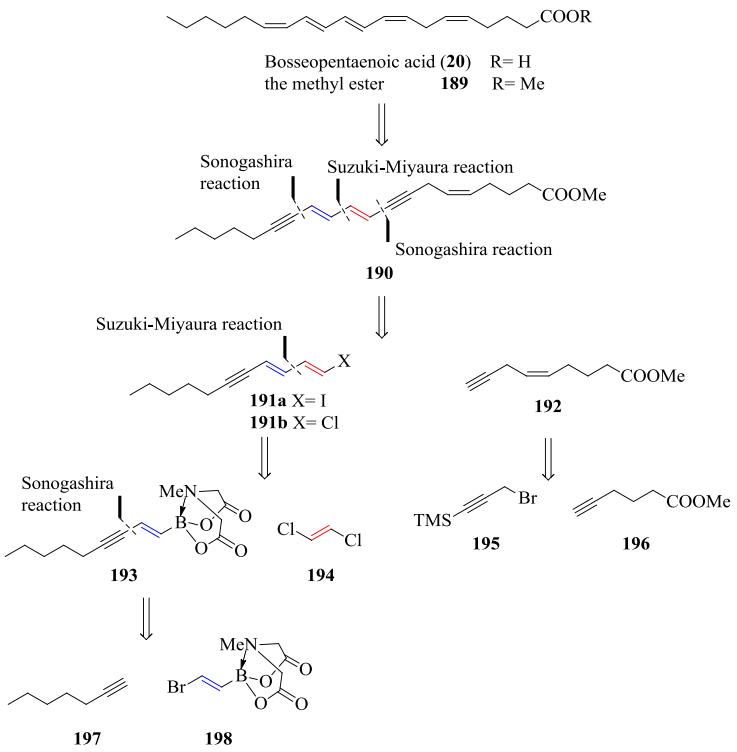


Arachidonic acid (**12**) 12-HETE (**37**) Bosseopentaenoic acid (**20**)

Scheme 2.9. Proposed biosynthetic pathway of bosseopentaenoic acid (**20**).

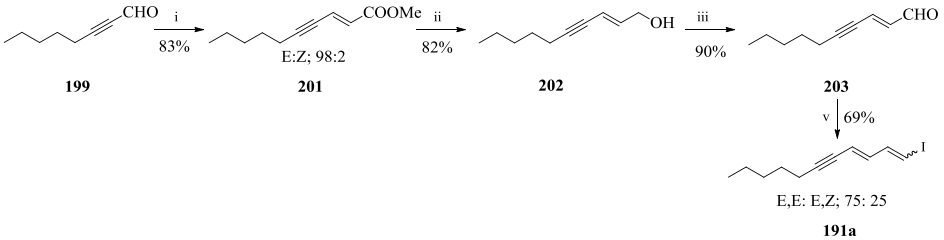
We decided to synthesize this fatty acid *via* consecutive palladium catalyzed cross coupling reactions. Our retrosynthetic analysis leads to the two building blocks **191** and **192**.

Compound **191b** (X= Cl) can be obtained from compound **193** which can be obtained from the MIDA boronate **198** developed by Burke and co-workers (Scheme 2.10).[33]



Scheme 2.10. Retrosynthetic analysis of bosseopentaenoicacid (**20**) and its methyl ester **189**.

Our initial strategy for obtaining the key intermediate **191a** (X= I) was a Wittig reaction between 2-octynal (**199**) and the stabilized ylide **200** to afford the adduct (*E:Z*, 98:2) **201** in 83% yield. This was reduced to the corresponding alcohol **202** in 82% yield using diisobutylaluminium hydride, and a subsequent oxidation provided **203**. Aldehyde **203** was used in a Takai reaction[34] to afford compound **191a** in 69% yield as a mixture of two isomers (*E*,*E*:*E*,*Z*, 75:25), as determined by ^{1}H NMR (Scheme 2.11).



i) Ph_3P=CHCOOMe (**200**), Toluene, 90 ^oC, 10 h ii) DIBAl-H, THF, rt, 3 h iii) MnO_2, CH_2Cl_2, rt, overnight
iv) $CrCl_3$, Zn, TMSCl, CHI_3,THF, rt, 4 h.

Scheme 2.11. Synthetic route for compound **191a**.

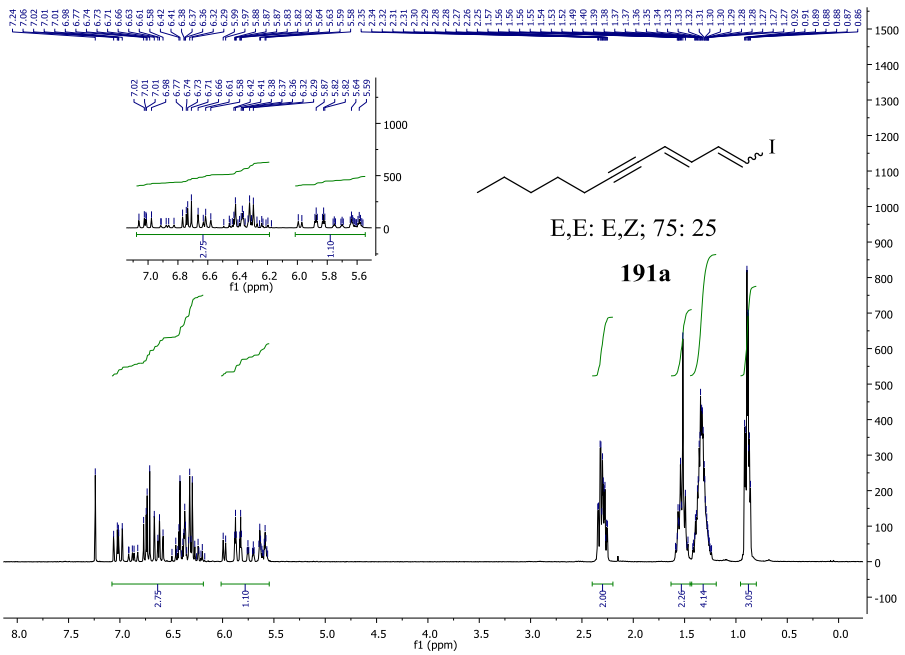
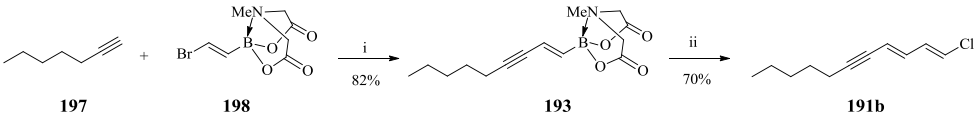


Figure 2.5. ^{1}H NMR for compound **191a**.

Since we could not obtain compound **191a** as a single isomer from the aforementioned described syntheses, we decided to prepare compound **191b** using the recently synthetic iterative protocol developed by Burke and co-workers.[33,35,36] The synthesis of compound **191b** (X=Cl) started by the formation of compound **193** *via* a Sonogashira reaction[26] of MIDA boronate **198** and alkyne **197**. The MIDA boronate **193** was obtained in 82% yield. This MIDA boronate is a precursor of its boronic acid after basic hydrolysis with 1N NaOH as previously reported.[33,35,36] From this point of view, we decided to react the Mida boronate **193** with *trans*-1,2-dichloroethene (**194**) in a Suzuki-Miyaura reaction[37] affording compound **191b**

in 70% yield. In contrast with the previous strategy, the produced compound **191b** was obtained as the *E*,*E*-isomer only (Scheme 2.12).



i) $Pd(Ph_3P)_2Cl_2$ (5 mol%), CuI (10 mol%), piperidine, THF, 3h; ii) *trans*-1,2 dichloroethene (**194**), $Pd(Ph_3P)_4$ (10 mol%), 1N aq. NaOH, THF, 60 ºC.

Scheme 2.12. Synthetic route for compound **191b**.

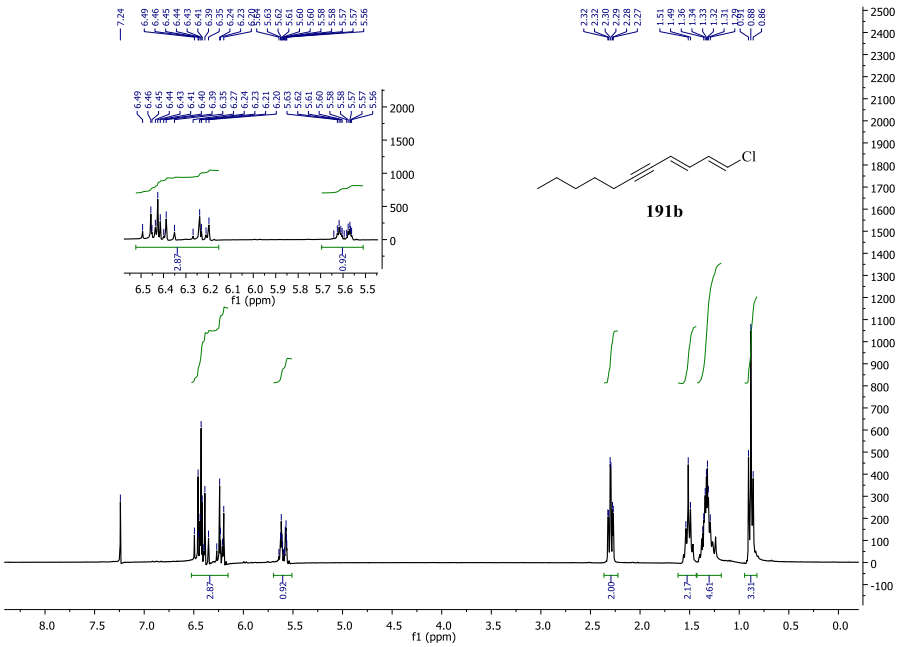
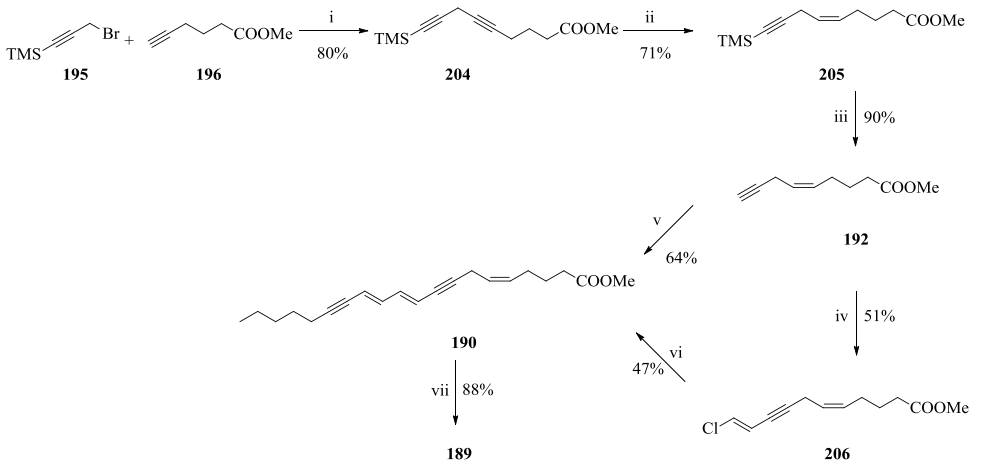


Figure 2.7. ^{1}H NMR for compound **191b**.

Alkyne **192** was synthesized according to a literature procedure.[38] The commercially available propargyl bromide **195** was reacted with alkyne **196** to obtain skipped alkyne **204** in 80% yield, followed by a stereoselective reduction of the internal triple bond using the Lindlar catalyst.[39] This gave compound **205** in 71% yield. Deprotection of the silyl group in compound **205** was carried out by treatment with potassium fluoride in methanol to afford compound **192** in 90% yield. This terminal alkyne was then reacted with *trans*-1,2-dichloroethene (**194**) in a Sonogashira reaction to afford compound **206** in 51% yield.

Hydrolysis of the MIDA boronate **193** under mild aqueous basic condition using 1 N NaOH to afford its boronic acid that was reacted with compound **206** *via* a Suzuki-Miyaura reaction to provide compound **190** in 47% yield. Due to the low yield of compound **190**, we decided to synthesize this compound by reacting compound **192** with compound **191b** *via* a Sonogashira reaction to afford diyne **190** in 64% yield. The diyne **190** was recovered when subjected to the reduction using Boland reduction procedure.[40] Then we tried to modify the Boland procedure by adding TMSCl to the reaction. This provided the methyl ester of bosseopentaenoic acid **189** in 88% (Scheme 2.13). This modification of Boland procedure was investigated in an extended study (Chapter 2.4).



i) CuI, *n*-Bu_4NBr, Na_2CO_3, DMF, rt, overnight; ii) H_2, Lindlar catalyst (50% wt), quinoline, MeOH; iii) KF, MeOH, 50 ^oC, 10 h; iv) *trans*-1,2-dichloroethene (**194**), $Pd(Ph_3P)_2Cl_2$ (1 mol %), CuI (10 mol%), piperidine, THF, rt, 4h; v) **191b**, $Pd(Ph_3P)_2Cl_2$ (5 mol%), CuI (10 mol%), piperidine, THF, rt, 6h; vi) (a) **193**, 1N aq. NaOH, THF, rt, 10 min, (b) $Pd(OAc)_2$ (2 mol%), SPhos, $K_3PO_4 \cdot 7H_2O$, THF-Toluene, 45 ^oC, 16h; vii) Zn(Cu/Ag), TMSCl, MeOH:H_2O (1:1, v:v), rt, 1h.

Scheme 2.13. Synthesis of bosseopentaenoic acid methyl ester **189**.

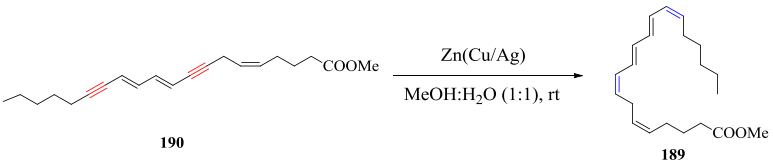
In conclusion, methyl (5*Z*,8*Z*,10*E*,12*E*,14*Z*)-eicosapentaenoate (**189**) was synthesized in 16% overall yield over seven steps. Our attempts to obtain acid **20** from ester **189** by hydrolysis under different basic conditions failed due to the sensitivity of compound **189** that either polymerized or decomposed under basic condition.

The results of this study have been summarized in **Paper III**, and the experimental procedure and spectroscopic data are attached in the appendix.

2.4. Paper (IV): *Z*-Stereoselective semi-reduction of alkynes: Modification of the Boland reduction protocol

The semi-reduction of alkynes to *cis*-alkenes is an important reaction in organic synthesis and in total synthesis of natural products.[41] The most popular catalyst used for *Z*-stereoselective semi-reduction of alkynes to *Z*-alkenes is the Lindlar catalyst.[39] However, this catalyst has some drawbacks, such as reproducibility and over-reduction to alkanes. Most serious is the problem with obtaining high *Z*-selectivity, especially in case of conjugated alkynes that lead to mixtures of *E*- and *Z*-isomers.[42] We have reported the first total synthesis of methyl (5*Z*,8*Z*,10*E*,12*E*,14*Z*)-eicosapentaenoate (**189**).[43] During this project we conducted some stereoselective reductions of alkynes to *cis*-alkenes using the Lindlar catalyst, but this catalyst failed to provide high *cis*-selectivity in conjugated systems. Hence, we needed to develop an improved protocol. For the reduction of the two triple bonds in compound **190** to obtain the natural product **189** (Scheme 2.14) we tried the Zn(Cu/Ag) system developed by Boland and coworkers.[40] However, only a 5% isolated yield was obtained after 24 hours at room temperature.

When we tried the Zn(Cu/Ag) system in presence of trimethylsilyl chloride, the reduction of **190** was highly accelerated. The methyl ester of bosseopentaenoic acid **189** was obtained with high *Z*-selectivity in excellent yield (Table 2.3).



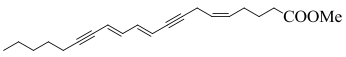
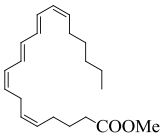
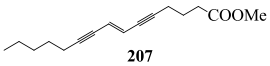
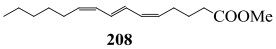
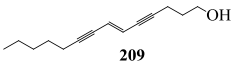
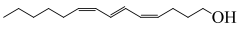
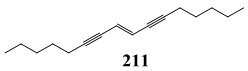
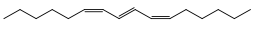
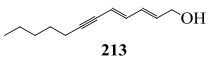
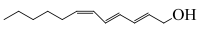
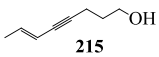
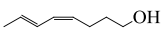
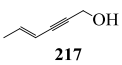
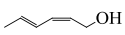
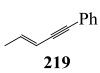
190 **189**

Scheme 2.14. Semi-reduction of compound **190**.

Amount of TMSCl	Reaction time	yield
without	24 h	5%
1 eq.	8 h	30%
5 eq.	4 h	82%
10 eq.	1 h	88%

Table 2.3. Amount of TMSCl added.

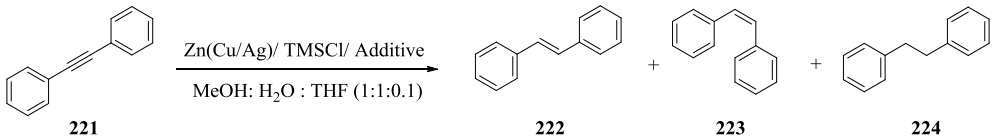
We have carried out this procedure with a variety of conjugated alkynes (Table 2.4).

Alkyne	Product	Time (min)	Diasteromeric ratio[a]	Yield (%)[b]
 **190**	 **189**	60	50:1	88
 **207**	 **208**	30	19:1	90
 **209**	 **210**	30	25:1	92
 **211**	 **212**	90	50:1	82
 **213**	 **214**	90	19:1	87
 **215**	 **216**	90	19:1	86
 **217**	 **218**	90	16:1	89
 **219**	 **220**	60	50:1	86

[a]:The diastereomeric ratios of the alkenes were determined by HPLC analysis. Two experiments were performed. The ratio is given of the diasteromer depicted compared to the sum of all other diasteromers formed. The structures of the minor diasteromers were not determined. [b]:The yields are of the *Z*-diasteromer depicted and determined after purification by chromatography and are the average of two experiments.

Table 2.4. Reduction of *E*-conjugated alkynes.

It is noteworthy that the Zn(Cu/Ag) system does not reduce non-activated alkynes such as diphenylacetylene (**221**). However, we aimed to investigate the effect of the additives on the Zn(Cu/Ag) system for this type of reduction (Table 2.5).



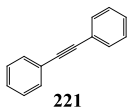
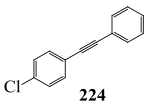
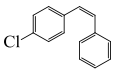
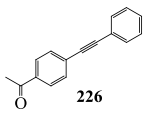
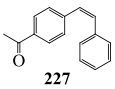
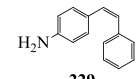
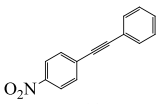
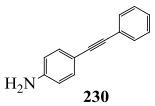
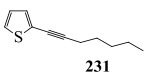
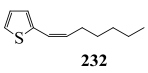
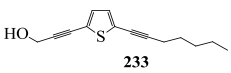
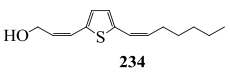
Scheme 2.15. Reduction of diphenylacetylene (**221**) as model substrate.

Entry	Additives	221: 222: 223: 224[c]	Time (h)	Isolated yield %[e]
1	without	-	36 h	-
2	TMSCl[a]	88.0: 0 : 12.0: 0	24 h	n.d.[d]
3	HCl[b]	49.2: 0 : 1.6 : 49.2	24 h	n.d.[d]
4	TMSCl /$ZnCl_2$	98.1 : 0 : 1.9 : 0	24 h	n.d.[d]
5	TMSCl /CuI	90.8: 0 : 9.2 : 0	24 h	n.d.[d]
6	TMSCl	79.2 : 0: 20.8: 0	36 h	28
7	TMSCl / $HSiEt_3$	35.0: 0 : 65.0 : 0	24 h	70
8	$HSiEt_3$	-	24 h	-

[a]:TMSCl (10 eq); [b]:1N HCl; [c]:Determined by GLC; [d]:not determined; [e]:Purified on silica gel (hexane).

Table 2.5. The effect of different additives.

We observed that the addition of TMSCl did not produce *cis*-stilbene **223** to a large extent. The reduction of alkyne **221** using the Zn(Cu/Ag) system was most efficient after adding both triethylsilane[44] and trimethylsilyl chloride (entry 7). The resulting product was only the *cis*-isomer **223** without any detection of the *trans*-isomer by ^{1}H NMR and GLC analyses. Moreover, adding triethylsilane alone (entry 8) yielded no conversion of 1,2-diphenylacetylene (**221**). Subsequently, we tested this newly investigated procedure on a variety of non-activated alkynes (Table 2.6).

Alkyne	Product	Time (h)	Diasteromeric ratio[a]	Yield (%)[b]
 **221**	 **223**	24	>98	70
 **224**	 **225**	24	>98	70
 **226**	 **227**	18	>98	72
	 **229** +	15	>98	54
 **228**	 **230**			30
 **231**	 **232**	15	>98	70
 **233**	 **234**	15	>98	60

[a]:The diastereomeric ratios of the alkenes were determined by GLC analysis. Two experiments were performed. The ratio is given of the*Z*-diasteromer. The structures of the minor diasteromers were not determined; [b]:The yields are of the *Z*-diasteromer determined after purification by chromatography and are the average of two experiments.

Table 2.6. Reduction of 1,2-diaryl alkynes and alkynylthiophene derivatives.

The use of $HSiEt_3$ as an additive with the Zn(Cu/Ag)/TMSCl leads to an efficient stereoselective semi-reduction of non-activated alkynes to *Z*-alkenes in good yields and high selectivity. This study adds a new value to the Boland reduction, since we have improved this

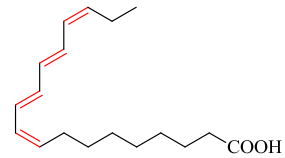
system to be efficient for several non-activated alkynes. A range of functional groups were tolerated in this procedure, such as chloro and acetyl. These functional groups were not affected under the reaction condition. However, the nitro group in compound **228** was reduced to amine under this reaction condition to afford (*Z*)-4-styrylaniline (**229**) and 4-(phenylethynyl)aniline (**230**) in 54% and 30% yields, respectively. The reduction of alkynyl thiophene to *Z*-alkenyl thiophene is not easy and isomeric mixtures of alkenes have often been reported.[45] The improved procedure was efficient for highly *Z*-selective semi-reduction of alkynyl thiophenes **231** and **233** affording *Z*-alkenyl thiophenes **232** and **234** in 70% and 60% yields, respectively (Table 2.6).

In conclusion, the Boland reduction procedure has been accelerated and improved by the addition of TMSCl. In this study, the synthesis of *Z*-alkenes could be achieved by hydrosilylation-protodesilylation process.[46] The mechanism of this reaction will be investigated in due course. The new modified reduction protocol facilitates the synthesis of sensitive or highly unsaturated polyene compounds, such as PUFAs or natural products that possess a polyene framework.

The results of this study have been described in **Paper IV** and the experimental procedure and spectroscopic data were attached in the appendix.

2.5. Synthetic studies towards α-parinaric acid

After the synthesis of the methyl ester of bosseopentaenoic acid **189**[43] and also after the development of the efficient procedure for stereoselective semi-reduction of alkynes to their corresponding *cis*-alkenes using the Zn(Cu/Ag)/TMSCl system,[47] we became interested in the synthesis of another polyene natural product. We therefore aimed to develop an efficient synthesis of α-parinaric acid (**19**), a conjugated tetraenoic fatty acid which possess the (*Z*,*E*,*E*,*Z*) moiety (Figure 2.8).



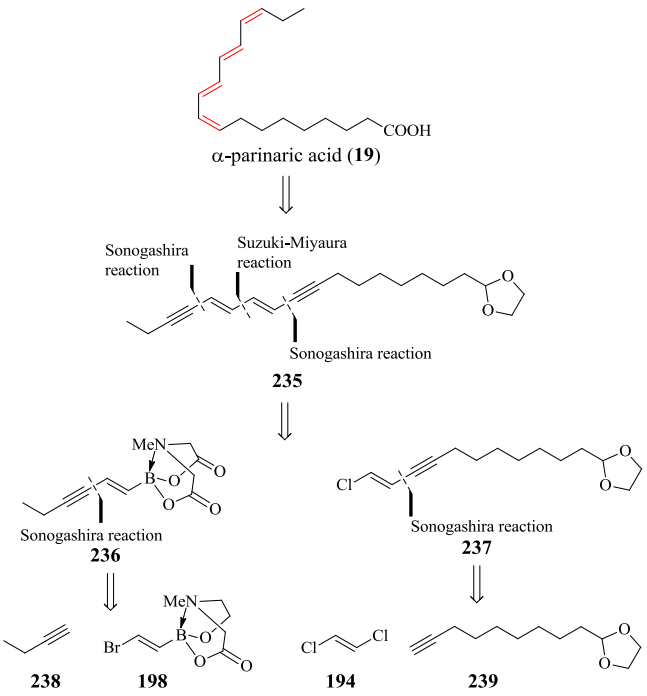
α-parinaric acid (**19**)
18:4 ω-3, *9Z*,11*E*,*13E*,15*Z*

Figure 2.8. The chemical structure of α-Parinaric acid (**19**).

α-Parinaric acid (**19**) was isolated by Tsujimoto and Koyanagi in 1933.[48] This natural product exhibit potent cytotoxic acidity[49] by sensitizing the tumor cells to lipid peroxidation, the process where free radicals react with electrons from cell membrane lipids, resulting in cell damage. It was reported that a mixture of three isomers of parinaric acids were prepared by bromination of α-linolenic acid and subsequent dehydrobromination.[50]

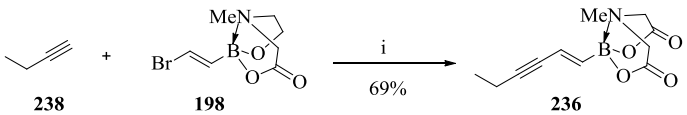
Due to the unique structure of α-parinaric acid (**19**) and its interesting bioactivity, we decided to synthesize this natural product by total synthesis.

Based on our published synthesis of methyl (5*Z*,7*Z*,9*E*,11*E*,13*Z*)-eicosapentaenoate (**189**),[43] the synthesis of α-parinaric acid (**19**) also depends on several palladium catalyzed cross coupling reactions. Our retrosynthetic analysis leads to compound **198** as the key starting material (Scheme 2.16).[35]



Scheme 2.16. Retrosynthetic analysis of α-parinaric acid (**19**).

Our synthetic strategy started with a Sonogashira coupling between 1-butyne (**238**) and MIDA boronate **198** to afford adduct **236** in 69% yield (Scheme 2.17).

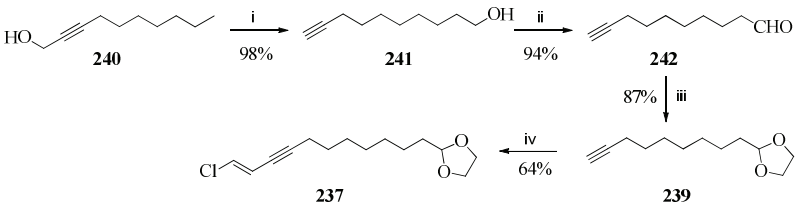


i) $Pd(Ph_3P)_2Cl_2$ (5 mol%), CuI (10 mol%), piperidine, THF, 3h.

Scheme 2.17.

The synthesis of compound **237** started with the synthesis of **241** from the commercially available alcohol **240** *via* an alkyne zipper reaction, transferring the internal alkyne to the terminal position, using literature procedure to afford alcohol **241**.[51] Oxidation of the alcohol **241** using the Dess-Martin periodinane reagent[52] afforded aldehyde **242** in 94% yield, which was then reacted with ethylene glycol in the presence of a catalytic amount of *p*-toluenesulphonic acid to give the corresponding acetal **239** in 87% yield. The Sonogashira

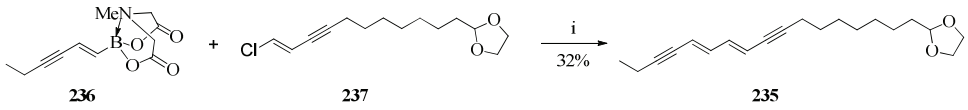
coupling between compound **239** and **194** afforded compound **237** in 64% yield (Scheme 2.18).



i) $H_2NCH_2CH_2CH_2NH_2Li$, *t*-BuOK, overnight; ii) Dess-Martin periodinane, 1.2 eq., CH_2Cl_2, 1.5h; iii) *p*-toluenesulphonic acid, 10 mol%, ethylene glycol, toluene, reflux, 8h; iv) **194**, $Pd(Ph_3P)_2Cl_2$ (5 mol%), CuI (10 mol%), piperidine, THF, 3h.

Scheme 2.18.

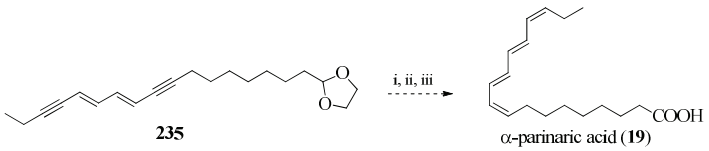
By the reaction of compound **237** with MIDA boronate **236** *via* a Suzuki-Miyauara cross coupling reaction, we obtained compound **235** in 32% yield (Scheme 2.19).



i) $Pd(Ph_3P)_4$ (10 mol%), 1N aq. NaOH (3 eq), THF, 60 °C, 2h.

Scheme 2.19.

The produced compound **235** can be a precursor for the synthesis of α-parinaric acid (**19**) by stereocontrolled reduction using the Zn(Cu/Ag)/TMSCl system.[47] Then deprotection of the acetal group to the aldehyde followed by oxidation using Pinnick oxidation method,[53] should yield the acid **19**.



i) stereoselective reduction by Zn(Cu/Ag)/TMSCl system;
ii) deprotection of acetal to aldehyde using HCOOH 80%, dioxane;
iii) oxidation of the aldehyde to the acid by Pinnick oxidation.

Scheme 2.20.

The experimental procedure and spectroscopic data of this study are attached in the appendix.

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3. Summary

An efficient synthesis of new polyunsaturated pyrroles has been reported. The new analogs were synthesized *via* a Stille coupling without using protecting groups for either the NH or the formyl group. The natural products mycalazol 5 (**55**) and mycalazal 2 (**56**) and the newly synthesized analogs were subjected to biological testing against different cancer cell lines. Mycalazol 5 (**55**) and mycalazal 2 (**56**) were the most active compounds; the two analogs possessing unsaturated alkyl chains showed slightly decreased cytotoxity than the natural products. Changing the pyrrole ring with a thiophene ring led to a further decrease in the cytotoxic activity.

An efficient synthesis of analogs of the polyunsaturated chromone **57** was reported. These new analogs exhibited potent antioxidant activity. It was observed that the compounds with free hydroxyl groups and polyunsaturated side chains exhibited antioxidative properties better than the other analogs.

The methyl ester of bosseopentaenoic acid (**189**) was synthesized *via* iterative palladium-catalyzed cross coupling using MIDA boronates in seven steps and in 16% overall yield.

The development of a highly *Z*-stereoselective semi-reduction method of alkynes to *Z*-alkenes was achieved by modification of the Boland reduction protocol. The diasteromeric ratios of the produced compounds ranged between 50:1 to 16:1. Also, this protocol was modified to the reduction of non-activated alkynes affording *Z*-stilbenes.

We have described synthetic studies towards α-parinaric acid (**19**) *via* iterative palladium-catalyzed cross coupling reactions. In this protocol, the construction of the conjugated diyne **235** has been achieved. This diyne is a precursor for the α-parinaric acid.

Appendix

Experimental section

General information

All reagents and solvents were used as purchased without further purification. Melting points were determined in open capillary tubes and are uncorrected. Analytical TLC was performed on silica gel 60 F_{254} Aluminium sheets (Merck). Flash column chromatography was performed on silica gel 60 (40-60 μm, Fluka). NMR spectra were recorded on a BrukerAvance DPX spectrometer at 300 or at 400 MHz for ^{1}H NMR, 75 or 101 MHz for ^{13}C NMR respectively. Coupling constants (*J*) are reported in hertz, and chemical shifts are reported in ppm (δ) relative to $CDCl_3$ (7.24 ppm for ^{1}H and 77.40 ppm for ^{13}C), DMSO-d_6 (2.49 ppm for ^{1}H and 39.5 ppm for ^{13}C) and pyridine-d_5 (8.71, 7.55, 7.19 for ^{1}H and 149.9, 135.5, 13.5 for ^{13}C). High resolution mass spectra were performed with VG Prospec mass spectrometer.

Procedures and spectral data

Eicosan-1-ol (178).

To a stirred solution of eicosanoic acid (**152**) (6.0 g, 16.78 mmol) in Et_2O (100 ml), fresh $LiAlH_4$ (0.869 g, 23.49 mmol) was added portionwise over 10 minutes at 0 °C. The mixture was stirred at 0 °C for 30 minute. Water (1 ml) was then added at 0 °C, followed by addition of 15% aq. NaOH (3.5 ml) and then water (1 ml) once more. The reaction mixture was dried over $MgSO_4$, filtered. The solvent was evaporated under reduced pressure to afford compound **178** in quantitative yield as white solid (5.50 g, 96%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.62 (t, J = 6.6 Hz, 2H), 1.59-1.50 (m, 2H), 1.31-1.24 (m, 34H), 0.86 (t, J = 6.6 Hz, 3H).^{13}C NMR (75 MHz, $CDCl_3$): δ 63.51, 33.23, 32.33, 30.09 (8 x CH_2), 30.06 (2 x CH_2), 30.01, 30.00, 29.83, 29.76, 26.14, 23.09, 14.51. HRMS Calcd. for $C_{20}H_{42}O$ $[M]^+$ 298.3236, found 298.3231.

Eicosanal (179).

Eicosanol (**178**) (5 g, 16.8 mmol) and silica gel (10 g, 166 mmol) were dissolved in CH_2Cl_2 (75 ml), followed by addition of pyridinium chlorochromate (5.4 g, 25.2 mmol). The reaction mixture was allowed to stir for 3 h at room temperature and then the mixture was filtered through a short plug of silica gel. The solvent was evaporated under reduced pressure to

afford a residue that was purified by column chromatography (silica gel, hexane/EtOAc, 9:1) to yield compound **179** as white solid (4.72 g, 95%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.74 (t, J = 1.8 Hz, 1H), 2.39 (td, J = 7.4, 1.9 Hz, 2H), 1.65-1.55 (m, 2H), 1.32-1.23 (m, 32H), 0.85 (t, J = 6.6 Hz, 3H).^{13}C NMR (75 MHz, $CDCl_3$): δ 203.28, 44.31, 32.32, 30.09 (8 x CH_2), 30.05, 30.02, 29.97, 29.82, 29.75, 29.56, 23.08, 22.49, 14.50. HRMS Calcd. for $C_{20}H_{40}O$ $[M]^+$ 296.3079, found 296.3074.

Heneicos-1-yne (180).

To a stirred mixture of zinc (1.2 g, 21.43 mmol) and triphenylphosphine (4.80 g, 18.32 mmol), carbon tetrabromide (6.10 g, 18.37 mmol) was stirred in CH_2Cl_2 (100 ml) for 40 h at room temperature. Aldehyde **179** (2.16 g, 7.29 mmol) in CH_2Cl_2 (20 ml) was added and stirred for 1 h at room temperature. The mixture was filtered through a short plug of silica gel. The filtrate was evaporated to yield pure dibromide as white solid (3.04 g, 92%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.36 (t, J = 7.2 Hz, 1H), 2.07 (q, J = 7.3 Hz, 2H), 1.24 (s, 34H), 0.86 (t, J = 6.6 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 139.29, 88. 84, 33.42, 32.34, 30.11(8 x CH_2), 30.08, 30.03, 29.93, 29.78, 29.75, 29.46, 28.21, 23.11, 14.53. To a solution of the dibromide (2 g, 4.54 mmol) in dry THF (60 ml), *n*-BuLi (3.47 ml, 5.56 mmol, 1.6 M in hexane) was added at -78 °C. The reaction mixture was allowed to stir at -78 ^{o}C for 1 h and then the mixture was allowed to warm at room temperature, followed by addition of water (50 ml). The mixture was extracted with hexane (2x50 ml). The combined organic layers was washed with brine (30 ml), dried ($MgSO_4$) and filtered. The solvent was evaporated under reduced pressure to afford a residue that was purified by column chromatography (silica gel, hexane) to yield alkyne **180** as white solid (1.18, 91%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.16 (td, J = 7.0, 2.6 Hz, 2H), 1.91 (t, J = 2.6 Hz, 1H), 1.55-1.46 (m, 2H), 1.39-1.24 (m, 30H), 0.86 (t, J = 6.6, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 85.21, 68.39, 32.32, 30.09 (8 x CH_2), 30.06, 30.01, 29.91, 29.76, 29.51, 29.17, 28.90, 23.09, 18.80, 14.51. HRMS Calcd. for $C_{21}H_{40}$ $[M]^+$ 292.3130, found 292.3130.

1-(2-Hydroxy-4,6-bis(methoxymethoxy)phenyl)ethanone (182b).

To a solution of 1-(2,4,6-trihydroxyphenyl) ethanone (**181b**) (1 g, 5.4 mmol) in acetone (10 ml), anhydrous K_2CO_3 (5.2 g, 40 mmol) was added, followed by addition of methoxy methyl bromide (1.68 g, 1.68 mmol). The mixture was heated at reflux for 12 h, then cooled to room temperature, filtered and evaporated under reduced pressure. The residue was purified by

column chromatography (silica, hexane/EtOAc, 7:3) to yield compound **182b** as white solid (0.76 g, 54%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 13.69 (s, 1H), 6.25 (d, J = 2.3 Hz, 1H), 6.22 (d, J = 2.3 Hz, 1H), 5.23 (s, 2H), 5.15 (s, 2H), 3.50 (s, 3H), 3.45 (s, 3H), 2.63 (s, 3H).^{13}C NMR (75 MHz, $CDCl_3$): δ 203.59, 167.22, 163.84, 160.74, 107.34, 97.55, 94.87, 94.41 (2 x CH), 57.10, 56.85, 33.41.

3-Iodo-5,7-bis(methoxymethoxy)-4H-chromen-4-one (184b).

Ketone **182b** (0.181 g, 0.70 mmol) and DMFDMA (0.1 ml, 0.76 mmol) was heated at 95 ^{o}C for 1.5 h. The reaction was then cooled at room temperature. The excess of DMFDMA was evaporated under reduced pressure using rotavapor. The residue was purified by column chromatography (silica gel, hexane/EtOAc, 1:1) to afford enaminone **183b** as yellow solid (0.192 g, 87%). To a stirred solution of **183b** (225 mg, 0.76 mmol) in MeOH (20 ml), iodine (260 mg, 1.02 mmol) was added as solid to the solution. The reaction mixture was allowed to stir overnight at room temperature. A solution of sat. aq. sodium thiosulfate (30 ml) was added and the mixture was extracted by $CHCl_3$ (3x50 ml). The organic solution was dried ($MgSO_4$), filtered and evaporated under reduced pressure. The resulting crude product was purified by column chromatography (silica gel, hexane/EtOAc, 1:1) to afford compound **184b** as white solid (0.251 g, 84%). m.p. 138-140 ^{o}C.^{1}H NMR (300 MHz, $CDCl_3$): δ 8.06 (s, 1H), 6.72 (d, J = 2.3 Hz, 1H), 6.68 (d, J = 2.3 Hz, 1H), 5.27 (s, 2H), 5.19 (s, 2H), 3.51 (s, 3H), 3.46 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.63, 161.90, 159.06, 158.52, 156.03, 109.11, 102.40, 97.11, 95.81, 94.75, 89.83, 57.11, 56.98.

3-((6*Z*,9*Z*,12*Z*,15*Z*,18*Z*)-Henicosa-6,9,12,15,18-pentaen-1-yn-1-yl)-7-hydroxy-4H-chromen-4-one (185a).

To a stirred solution of compound **184a** (1.116 g, 3 mmol), CuI (114 mg, 0.6 mmol) and $Pd(Ph_3P)_2Cl_2$ (63 mg, 0.09 mmol) in THF (9 ml), triethylamine (1.25 ml, 9.0 mmol) was added, followed by addition of alkyne **122** (1.27 g, 4.5 mmol). The reaction mixture was allowed to stir at room temperature for 2 h. The mixture was then diluted with EtOAc (20 ml) and washed with sat. aq. NH_4Cl. The organic solution was dried over $MgSO_4$, filtered and evaporated under reduced pressure. The resulting crude product was dissolved in MeOH/THF (1:1, 180 ml), followed by addition of *p*-toluenesulfonic acid monohydrate (95 mg, 0.5 mmol). This mixture was stirred at 60 °C for 1 h and then allowed to cool to room temperature. Et_3N (0.69 ml, 5.0 mmol) was added. The mixture was evaporated to afford a

residue that was purified by column chromatography (silica gel, hexane/EtOAc, 7:3) to give compound **185a** as brown oil (1.08 g, 82%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.20 (br s, 1H), 8.11 (d, *J* = 8.8 Hz, 1H), 8.03 (s, 1H), 7.24 (s, 1H), 7.09 (dd, *J* = 8.8, 2.2 Hz, 1H), 6.97 (d, *J* = 2.2 Hz, 1H), 5.44-5.19 (m, 10H), 2.79-2.75 (m, 8H), 2.37 (t, *J* = 7.2 Hz, 2H), 2.16 (q, *J* = 6.9 Hz, 2H), 2.04 (p, *J* = 7.2 Hz, 2H), 1.62 (p, *J* = 7.3 Hz, 2H), 0.94 (t, *J* = 7.5 Hz, 3H).^{13}C NMR (75 MHz, $CDCl_3$): δ 177.06, 163.58, 158.25, 157.92, 132.14, 129.08, 128.89, 128.67, 128.43, 128.35, 128.21, 128.20, 127.98, 127.73, 127.13, 116.58, 116.17, 111.04, 103.21, 97.02, 70.52, 28.43, 26.54, 25.77, 25.76, 25.73, 25.66, 20.68, 19.26, 14.39. HRMS calcd. for $C_{30}H_{34}O_3$ $[M]^+$ 442.2508, found 235.1011.

3-((6*Z*,9*Z*,12*Z*,15*Z*,18*Z*)-heneicosa-6,9,12,15,18-pentaen-1-yn-1-yl)-7-methoxy-4H-chromen-4-one (185b).

To a stirred mixture of **185a** (100 mg, 0.226 mmol) and anhydrous K_2CO_3 (47 mg, 0.339 mmol) in dry acetone (10 ml), MeI (48 mg, 0.339 mmol) was added. The mixture was heated at reflux for 2 h and then allowed to cool to room temperature. The mixture was filtered and evaporated under reduced pressure to afford a residue that was purified by column chromatography (silica gel, hexane/EtOAc, 8:2) to afford **185b** as yellow oil (82 mg, 80%). ^{1}H NMR (300 MHz, $CDCl_3$): δ8.12 (d, *J* = 8.9 Hz, 1H), 7.97 (s, 1H), 6.93 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.78 (d, *J* = 2.4 Hz, 1H), 5.45-5.21 (m, 10H), 3.87 (s, 3H), 2.89-2.74 (m, 8H), 2.44 (t, *J* = 7.2 Hz, 2H), 2.22 (q, *J* = 7.1 Hz, 2H), 2.04 (p, *J* = 7.1 Hz, 2H), 1.67 (p, *J* = 7.2 Hz, 2H), 0.94 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 175.23, 164.24, 157.80, 157.20, 132.14, 129.19, 128.91, 128.66, 128.53, 128.33, 128.26, 128.18, 128.01, 127.75, 127.14, 117.58, 114.89, 111.70, 100.42, 96.13, 71.00, 55.96, 28.59, 26.53, 25.80, 25.77, 25.73, 25.66, 20.68, 19.34, 14.40. HRMS calcd. for $C_{31}H_{36}O_3$ $[M]^+$ 456.2664, found 456.2660.

3-(Heneicos-1-yn-1-yl)-7-hydroxy-4H-chromen-4-one (186a)

To a stirred solution of compound **184a** (0.323 g, 0.87 mmol), CuI (40 mg, 0.20 mmol), $Pd(Ph_3P)_2Cl_2$ (14 mg, 0.03 mmol) in THF (9 ml), triethylamine (0.42 ml, 3.0 mmol) was added, followed by addition of alkyne **180** (0.29 g, 1.00 mmol). The reaction mixture was allowed to stir at room temperature for 2 h. The mixture was then diluted with EtOAc (20 ml) and washed with satd. aq. NH_4Cl. The organic solution was dried ($MgSO_4$), filtered and evaporated under reduced pressure. The crude product was dissolved in MeOH/THF (1:1, 180 ml), followed by addition of *p*-toluenesulfonic acid monohydrate (4 mg, 0.021 mmol). This

mixture was stirred at 60 °C for 1 h and then allowed to cool to room temperature. Et_3N (0.03 ml, 0.21 mmol) was added. The mixture was evaporated to afford a residue that was purified by column chromatography (silica gel, hexane/EtOAc, 8.5:1.5) to yield compound **186a** as white solid (0.314 g, 80%). ^{1}H NMR (300 MHz, Pyr-d_5): δ 8.39 (s, 1H), 8.33 (d, J = 8.8 Hz, 1H), 7.14 (dd, J = 8.8, 2.3 Hz, 1H), 7.00 (d, J = 2.2 Hz, 1H), 4.98 (br s, 1H), 2.42 (t, J = 7.0 Hz, 2H), 1.56 (dt, J= 14.0, 6.4 Hz, 2H), 1.28-1.24 (m, 32H), 0.85 (t, J = 6.6 Hz, 3H).^{13}C NMR (75 MHz, Pyr-d_5): δ 175.09, 164.54, 158.44, 158.26, 128.05, 117.04, 116.39, 111.80, 103.47, 96.14, 72.64, 32.28 , 30.16(8 x CH_2), 30.08 (2 x CH_2), 29.97, 29.77, 29.60, 29.33, 29.11, 23.09, 20.01, 14.43. HRMS calcd. for $C_{30}H_{44}O_3$ $[M]^+$ 452.3290 found 452.3287.

3-(heneicos-1-yn-1-yl)-7-methoxy-4H-chromen-4-one (186b)

To a stirred mixture of **186a** (20 mg, 0.044 mmol) and anhydrous K_2CO_3 (9 mg, 0.066 mmol) in acetone (10 ml), MeI (9 mg, 0.066 mmol) was added. The mixture was heated at reflux for 2 h. The resulting mixture was then cooled to room temperature, filtered and evaporated under reduced pressure. The residue was purified by column chromatography (silica gel, hexane/EtOAc, 8:2) to yield compound **186b** (18 mg, 87%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.12 (d, J = 8.9 Hz, 1H), 7.98 (s, 1H), 6.94 (dd, J= 8.9, 2.3 Hz, 1H), 6.78 (d, J = 2.4 Hz, 1H)3.87 (s, 3H), 2.41 (t, , J = 7.1 Hz, 2H), 1.63-1.54 (m, 2H), 1.26-1.23 (m, 32H), 0.85 (t,, J = 6.6 Hz, 3H).^{13}C NMR (75 MHz, $CDCl_3$): δ 175.58, 164.51, 158.09, 157.47, 128.04, 117.86, 115.16, 112.06, 100.68, 96.88, 70.90, 56.23, 32.32, 30.10 x 8 CH_2, 30.06 x 2 CH_2, 29.92 , 29.76 , 29.57, 29.38, 29.01, 23.09, 20.13, 14.51. HRMS calcd. for $C_{31}H_{46}O_3$ $[M]^+$ 466.3447, found 466.3443.

3-((6*Z*,9*Z*,12*Z*,15*Z*,18*Z*)-heneicosa-6,9,12,15,18-pentaen-1-yn-1-yl)-5,7-dihydroxy-4H-chromen-4-one (187a).

To a stirred solution of compound **184b** (0.260 g, 0.67 mmol), CuI (23 mg, 0.12 mmol), $Pd(Ph_3P)_2Cl_2$ (21 mg, 0.03 mmol) in THF (9 ml), triethylamine (0.28 ml, 1.98 mmol) was added, followed by addition of alkyne **122** (0.28 g, 1.00 mmol). The reaction mixture was allowed to stir at room temperature for 2 h. The mixture was diluted with EtOAc (20 ml) and washed with sat. aq. NH_4Cl. The organic solution was dried over $MgSO_4$, filtered and evaporated under reduced pressure. The resulting crude product was then dissolved in $CHCl_3$ (20 ml), MeOH (10 ml), followed by addition of conc. HCl (1 ml). The mixture was refluxed for 1 h. The reaction was then quenched with water and extracted with $CHCl_3$ (3 x 15 ml).

The combined organic layers was dried ($MgSO_4$), filtered and evaporated under reduced pressure to afford a residue that was purified by column chromatography (silica gel, hexane/EtOAc, 7:3) to yield **187a** as yellow oil (0.243 g, 80%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 12.39 (s, 1H), 7.93 (s, 1H), 6.84 (s, 1H), 6.37 (d, J = 2.2 Hz, 1H), 6.32 (d, J = 2.2 Hz, 1H), 5.42-5.22 (m, 10H), 2.83-2.75 (m, 8H), 2.39 (t, J = 7.2 Hz, 1H), 2.18 (q, J = 7.0 Hz, 2H), 2.03 (p, J = 7.0 Hz, 2H), 1.63 (p, J = 7.2 Hz, 2H), 0.95 (t, J = 7.5 Hz, 1H).^{13}C NMR (75 MHz, $CDCl_3$): δ 180.82, 163.39, 162.72, 158.32, 158.09, 132.47, 129.32, 129.28, 128.98, 128.75, 128.67, 128.52 (2 x CH), 128.29, 127.44, 110.40, 105.66, 100.56, 97.81 , 95.00, 69.75, 28.71, 26.80, 26.06 (2 x CH_2), 26.03, 25.95, 20.98, 19.53, 14.69. HRMS calcd. for $C_{30}H_{34}O_4$ $[M]^+$ 458.2457, found 458.2455.

3-((6*Z*,9*Z*,12*Z*,15*Z*,18*Z*)-henicosa-6,9,12,15,18-pentaen-1-yn-1-yl)-5-hydroxy-7-methoxy-4H-chromen-4-one (187b).

To a stirred mixture of **187a** (30 mg, 0.066 mmol) and anhydrous K_2CO_3 (14 mg, 0.10 mmol) in acetone (3 ml) 1 ml, MeI (14 mg, 0.10 mmol) was added. The mixture was heated at reflux for 2 h and it was subsequently cooled to room temperature, filtered and evaporated under reduced pressure. The resulting crude product was purified by column chromatography (silica gel, hexane/EtOAc, 7:3) to yield compound **187a** as yellow oil (24 mg, 83 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ 12.46 (s, 1H), 7.93 (s, 1H), 6.34 (s, 2H), 5.38-5.34 (m, 10H), 3.83 (s, 3H), 2.81-2.75 (m, 8H), 2.45 (t, J = 7.1 Hz, 2H), 2.22 (q, J = 6.7 Hz, 2H), 2.05 (p, J = 7.1 Hz, 2H), 1.67 (p, J = 7.2 Hz, 2H), 0.95 (t, J = 7.5 Hz, 3H).^{13}C NMR (75 MHz, $CDCl_3$): δ 180.55, 166.14, 162.70, 158.02, 157.91, 132.45, 129.35, 129.33, 128.97, 128.76, 128.66, 128.51 x 2CH, 128.29, 127.43, 110.54, 105.87, 98.93, 97.29, 93.27, 70.02 , 56.26, 28.81, 26.79, 26.09, 26.06, 26.02, 25.95, 20.97, 19.56, 14.69. HRMS calcd. for $C_{31}H_{36}O_4$ $[M]^+$ 472.2614, found 472.2605.

3-(Henicos-1-yn-1-yl)-5,7-dihydroxy-4H-chromen-4-one (188a).

To a stirred solution of compound **184b** (0.26 g, 0.67 mmol), CuI (23 mg, 0.12 mmol), $Pd(Ph_3P)_2Cl_2$ (21 mg, 0.03 mmol) in THF (9 ml), triethylamine (0.28 ml, 1.98 mmol) was added, followed by addition of alkyne **180** (0.29 g, 1.00 mmol). The reaction mixture was allowed to stir at room temperature for 2 h. The mixture was then diluted with EtOAc (20 ml) and washed with sat. aq. NH_4Cl. The organic solution was dried over $MgSO_4$, filtered and evaporated under reduced pressure using rotavapor. The resulting crude product was

dissolved in CH_3Cl (20 ml), MeOH (10 ml), followed by addition of conc. HCl (1 ml). The mixture was refluxed for 1 h and then quenched with water. The mixture was then extracted with $CHCl_3$ (3x15 ml). The combined organic layers was dried ($MgSO_4$), filtered and evaporated under reduced pressure using to afford a residue that was purified by column chromatography (silica gel, hexane/EtOAc, 7:3) to afford compound **188a** as white solid (0.258 g, 83%). ^{1}H NMR (300 MHz, Pyr-d_5): δ 13.26 (s, 1H), 8.42 (s, 1H), 6.70 (d, J = 2.1 Hz, 1H), 6.60 (d, J = 2.1 Hz, 1H), 5.04 (brs, 1H), 2.45 (t, J = 6.9 Hz, 2H), 1.62-1.55 (m, 2H), 1.32-1.30 (m, 32H), 0.89 (t, J = 6.6 Hz, 3H). ^{13}C NMR (75 MHz, Pyr-d_5): δ 180.56, 166.46, 163.21, 159.13, 158.37, 123.97,110.25, 105.08, 100.65, 97.00, 95.21, 71.20, 32.28, 30.16(8 x CH_2), 30.08, 29.96, 29.77, 29.57, 29.27, 29.00, 23.09, 19.96, 14.43. HRMS calcd. for $C_{30}H_{44}O_4$ $[M]^+$ 468.3240, found 468.3237.

3-(Henicos-1-yn-1-yl)-5-hydroxy-7-methoxy-4H-chromen-4-one (188b)

To a stirred mixture of **188a** (30 mg, 0.064 mmol) and anhydrous K_2CO_3 (13 mg, 0.09 mmol) in acetone (3 ml), MeI (13 mg, 0.09 mmol) was added. The mixture was heated at reflux for 2 h. The reaction mixture was then cooled to room temperature, filtered and evaporated under reduced pressure. The residue was purified by column chromatography (silica gel, hexane/EtOAc, 8:2) to yield compound **188b** as yellow oil (31 mg, 90%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 12.46 (s, 1H), 7.93 (s, 1H), 6.34 (s, 2H), 3.83 (s, 3H), 2.42 (t, J = 7.2 Hz, 2H), 1.64-1.53 (m, 2H), 1.28-1.23 (m, 32H), 0.85 (t, J = 6.6 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 180.55, 166.09, 162.65, 158.07, 157.88, 110.60, 105.84, 98.89 , 97.71, 93.22, 69.63, 56.22, 32.32, 30.10(8 x CH_2), 30.06, 30.03, 29.91, 29.76, 29.54, 29.37, 28.95, 23.09, 20.07, 14.51. HRMS calcd.for $C_{31}H_{46}O_4$ $[M]^+$ 482.3396, found 482.3393.

(*E*)-methyl dec-2-en-4-ynoate (201).

2-Octynal (**199**) (1 g, 8.06 mmol) and Wittig reagent **200** (3.24 g, 9.72 mmol) in dry toluene (10 ml) under argon was heated at 90 ^{o}C for 10 h. The reaction mixture was cooled at room temperature. The solvent was evaporated under reduced pressure to afford a residue that was treated with Et_2O (20 ml), filtered and the solvent evaporated. The resulting crude product was purified by column chromatography (silica gel, hexane/EtOAc, 98:2) to yield compound **201** as yellow oil (1.20 g, 83%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.74 (dt, J = 15.8, 2.3 Hz, 1H), 6.12 (d, J = 15.8 Hz, 1H), 3.72 (s, 3H), 2.34 (td, J = 7.1, 2.2 Hz, 2H), 1.52 (td, J = 7.1 Hz, 2.2 Hz, 2H), 1.33-1.27 (m, 4H), 0.88 (t, J = 7.0 Hz, 3H).^{13}C NMR (75 MHz, $CDCl_3$):

δ166.98, 129.04, 126.70, 101.55, 78.28, 52.09, 31.43, 28.39, 22.54, 20.13, 14.31.HRMS calcd. for $C_{11}H_{16}O_2$ $[M]^+$180.1150, found 180.1147.

(*E*)-dec-2-en-4-yn-1-ol (202).

To a solution of compound **201** (0.315 g, 1.75 mmol) in dry THF (30 ml) under argon, diisobutyl aluminium aluminum hydride (3.5 ml, 1.0 M, 3.5 mmol) was added at room temperature over a period 15 min at 0 ^oC. The reaction mixture was allowed to warm at ambient temperature and stirred at this temperature for 3 h. The reaction was then quenched by adding 1M HCl (6 ml). The mixture allowed to stir for 30 min and then extracted with Et_2O (3x50 ml) to afford a residue that was purified by column chromatography (silica gel, hexane/EtOAC, 97:3) to yield compound **202** as yellow oil (0.22 g, 82%).^{1}H NMR (300 MHz, $CDCl_3$): δ 6.13 (dt, J = 15.8, 5.5 Hz, 1H), 5.69 (dt, J = 15.8, 2.0 Hz, 1H), 4.15 (dd, J = 5.5, 1.7 Hz, 2H), 2.27 (td, J = 7.1, 2.2 Hz, 2H), 1.54-1.48 (m, 4H), 1.34-1.31 (m, 4H), 0.88 (t, J = 7.1 Hz, 3H). ^{13}C NMR: δ (75 MHz, $CDCl_3$) δ 140.48, 111.77, 91.89, 78.65, 63.48, 31.47, 28.80, 22.60, 19.75, 14.36. HRMS calcd. for $C_{10}H_{16}O$ $[M]^+$ 152.1201, found 152.1200.

(*E*)-dec-2-en-4-ynal (203).

To a solution of alcohol (0.146 g, 0.9 mmol) in dichloromethane (30 ml), manganese dioxide (1.67 g, 18 mmol) was added. The reaction mixture was allowed to stir at room temperature overnight and then the mixture was filtered. The solvent was evaporated to afford a residue that was purified by column chromatography (silica gel, hexane/EtOAc, 97:3) to yield compound **203** as yellow oil (85 mg, 90%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.52 (d, J = 7.8 Hz, 1H), 6.58 (dt, J = 15.8, 2.2 Hz, 1H), 6.36 (dd, J = 15.8, 7.8 Hz, 1H), 2.41 (td, J = 7.1, 2.2 Hz, 2H), 1.61-1.52 (m, 2H), 1.37-1.33 (m, 4H), 0.89 (t, J = 7.0 Hz, 3H).^{13}C NMR (75 MHz, $CDCl_3$): δ 193.89, 139.35, 134.31, 107.82, 74.81, 31.44, 28.27, 22.53, 20.43, 14.31.HRMS calcd. for $C_{10}H_{14}O$ $[M]^+$ 150.1045, found 150.1041.

(1*E*,3*E*)-1-iodoundeca-1,3-dien-5-yne (191a).

To a mixture of $CrCl_3$ (26mg, 0.165 mmol) and zinc (0.257 g, 3.96 mmol) in dry THF (7 ml), TMSCl (0.43 ml, 3.96 mmol) was added. The reaction mixture was allowed to stir at room (ca 40 min) until the color of the solution turned to green, which was an indication of the formation of $CrCl_2$. A mixture of aldehyde **203**(49 mg, 0.33 mmol) and iodoform (0.26 g, 0.66 mmol) in dry THF (3 ml) was added to the reaction mixture dropwise over a period 1.5

h. The reaction mixture became brown and was stirred at room temperature for 4 h. The reaction was diluted with hexane (10 ml), water (50 ml) was added. The organic layer was collected and then the aqueous layer was extracted with Et_2O (3x50 ml). The combined organic layers was dried ($MgSO_4$), filtered and evaporated to afford a residue that was purified by column chromatography (silica gel, hexane) to afford compound **191a** as yellow oil (54 mg, 69%) yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.12-6.14 (m, 3H), 6.03-5.52 (m, 1H), 2.30 (dtd, J = 12.0, 7.1, 2.3 Hz, 2H), 1.59-1.47 (m, 2H), 1.42-1.47 (m, 4H), 0.89 (td, J = 7.0, 3.1 Hz, 3H).^{13}C NMR (75 MHz, $CDCl_3$): δ 144.98, 140.20, 139.83, 138.29, 117.06, 113.46, 96.96, 83.95, 31.51, 28.69, 22.60, 20.16, 14.36. HRMS calcd. for $C_{11}H_{15}I$ $[M]^+$274.0218, found 274.0207.

(*E*)-2-(Hex-1-en-3-yn-1-yl)-6-methyl-1,3,6,2-dioxazaborocane-4,8-dione (236).

To a mixture of (*E*)-2-Bromovinyl MIDA boronate (201) (0.2 g, 0.77 mmol), $Pd(Ph_3P)_2Cl_2$ (30 mg, 0.04 mmol), CuI (90 mg, 0.47mmol,) in dry THF (20 ml) under argon, piperidine (0.3 ml, 3 mmol) was added, followed by the addition of condensed 1-butyne **238** (0.6 ml, 7.7mmol). The mixture was allowed to stir at room temperature for 3 h. The resulting mixture was diluted with EtOAc (10 ml) then filtered through short pad of silica gel using EtOAc (50 ml) as eluent. The solvent was evaporated to afford a residue that was purified by column chromatography (silica gel, hexane: EtOAc, 1:1), followed by Et_2O and EtOAc) to yield compound **236** as colorless solid (0.116 g, 69 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.16-5.85 (m, 2H), 3.83 (d, J = 16.3 Hz, 2H), 3.66 (d, J = 16.4 Hz, 2H), 2.84 (s, 3H), 2.31 (qd, J = 7.5, 2.0 Hz, 2H), 1.14 (t, J = 7.5 Hz, 3H).^{13}C NMR (101 MHz, $CDCl_3$): δ 166.79, 125.18, 94.37, 79.65, 61.42, 46.72, 13.74, 13.11. HRMS calcd. for $C_{11}H_{14}BNO_4$ $[M]^+$ 235.1016, found 235.1011.

Dec-9-yn-1-ol (241).

Lithium (0.272 g, 38.97 mmol, 6.0 equivalents) was dissolved in 1,3-diaminopropane (30 ml) and stirred for 30 min at room temperature. The reaction mixture was allowed to stir at 70 ^{o}C until the blue color had disappeared and afforded a white suspension of the lithium amide. *t*-BuOK (2.94 g, 26 mmol, 4.0 equivalents) was added. The formed yellow solution was stirred for 20 min at room temperature and then 2-decyne-1-ol **240** (1 g, 6.5 mmol, 1.0 equivalent) was added to the reaction mixture over 10 min at room temperature. The reddish brown mixture was allowed to stir overnight at room temperature. The reaction mixture was

poured into ice/water (200 ml), extracted with Et_2O (3 x 50 ml) and washed with water (50 ml), 10% HCl (50 ml) and brine (50 ml). The organic solution was dried ($MgSO_4$). The solvent was evaporated to yield alkyne **241** (0.98 g, 98%), without any further purification.^{1}H NMR (400 MHz, $CDCl_3$): δ 3.61 (t, J = 6.6 Hz, 2H), 2.16 (td, J = 7.0, 2.6 Hz, 2H), 1.91 (t, J = 2.7 Hz, 1H), 1.51 (dq, J = 14.8, 7.3 Hz, 4H), 1.46-1.18 (m, 8H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 84.75, 68.09, 63.03, 32.76, 29.27, 29.05, 28.66, 28.45, 25.68, 18.39. HRMS calcd. for $C_{10}H_{18}O$ $[M]^+$ 154.1358, found 154.1355.

Dec-9-ynal (242).

To a stirred solution of alcohol **241** (0.5 g, 3.25 mmol, 1.0 eq) in dichloromethane (30 ml), Dess-Martin periodinane (1.65 g, 3.9 mmol, 1.2 eq) was added. The reaction was allowed to stir at room temperature for 1.5 h and then diluted with EtOAc (20 ml). The reaction mixture was washed with a mixture of sat. aq. $NaHCO_3$ and 10% aq. sodium thiosulfate (1:1, 10 ml). The aqueous layer was extracted with EtOAc (2 x 30 ml). The combined solvent was washed with water, dried ($MgSO_4$). The solvent was filtered and evaporated to afford a residue that was purified by column chromatography (silica gel, 15% EtOAc in hexane) to yield compound **242** as colorless oil (0.46 g, 94 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.74 (s, 1H), 2.40 (td, J = 7.4, 1.8 Hz, 2H), 2.15 (dt, J = 6.8, 3.5 Hz, 2H), 1.91 (t, J = 2.7 Hz, 1H), 1.70-1.17 (m, 12H).^{13}C NMR (101 MHz, $CDCl_3$): δ 202.85, 84.61, 68.18, 43.87, 29.00, 28.82, 28.48, 28.35, 21.99, 18.35. HRMS calcd. for $C_{10}H_{18}O$ $[M]^+$152.1201, found 152.1118.

2-(Non-8-yn-1-yl)-1,3-dioxolane (239).

Aldehyde **242** (0.40 g, 2.6 mmol) in toluene (15 ml) was treated with ethylene glycol (0.30 ml, 5.65 mmol, 2.15 eq) and a catalytic amount of *p*-TsOH. The mixture was refluxed for 8 h with a Dean-Stark trap and poured into a cold saturatedaqueous $NaHCO_3$ (50 mL). The organic layer was separated, washed with brine (10 ml), dried ($MgSO_4$), and filtered. The solvent was evaporated under reduced pressure, and the resulting crude product was purified by column chromatography (silica gel, hexane/EtOAc, 97:3) to yield compound **239** as colorless oil (0.45 g, 87%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.81 (t, J = 4.8 Hz, 1H), 4.01-3.73 (m, 4H), 2.14 (td, J = 7.1, 2.6 Hz, 2H), 1.90 (t, J = 2.6 Hz, 1H), 1.63-1.55 (m, 2H), 1.55-1.25 (m, 10H).^{13}C NMR (101 MHz, $CDCl_3$): δ 104.65, 84.71, 68.06, 64.82, 33.87, 29.37, 28.98, 28.61, 28.44, 24.00, 18.36.HRMS calcd. for $C_{12}H_{20}O_2$ $[M]^+$ 196.1463, found 196.1457.

(*E*)-2-(11-chloro-undec-10-en-8-yn-1-yl)-1,3-dioxolane (237).

*trans*1,2-Dichloroethene **194** (0.94 ml, 1.80 mmol, 5 eq) and piperidine (0.57 ml, 5.86 mmol, 3 eq) were added to a mixture of $Pd(Ph_3P)_2Cl_2$ (68 mg, 0.097 mmol, 5 mol%) and CuI (74 mg, 0.39 mmol, 20 mol%) in dry THF (10 ml) under argon. Subsequently, a solution of 2-(non-8-yn-1- yl)-1,3-dioxolane (**239**) (0.38 g, 1.95 mmol) in dry THF (1 ml) was added. The reaction mixture was allowed to stir for 3 h at room temperature. The resulting mixture was then diluted with EtOAc (20 ml) then filtered through short pad of silica gel using EtOAc (50 ml) as eluent. The filtrate was evaporated under reduced pressure and the crude product was purified by column chromatography using (silica gel, hexane/EtOAc, 97:3) to yield compound **237** as a colorless oil (0.32 g, 64%).^{1}H NMR (400 MHz, $CDCl_3$): δ 6.51-6.41 (m, 1H), 5.59 (dd, J = 11.9, 2.7 Hz, 1H), 4.82 (t, J = 4.9 Hz, 1H), 3.93 (d, J = 6.7 Hz, 2H), 3.83 (d, J = 6.7 Hz, 2H), 2.30 (td, J = 7.1, 2.0 Hz, 2H), 1.63 (dt, J = 8.7, 5.2 Hz, 2H), 1.49 (q, J = 7.1 Hz, 2H), 1.41-1.26 (m, 8H).^{13}C NMR (101 MHz, $CDCl_3$): δ 139.63, 113.19, 104.66, 94.79, 79.88, 64.83, 33.88, 29.38, 29.01, 28.75, 28.65, 24.01, 19.67. HRMS calcd. for $C_{14}H_{21}ClO_2$ $[M]^+$ 256.1230, found 256.1224.

2-((10*E*,12*E*)-heptadeca-10,12-dien-8,14-diyn-1-yl)-1,3-dioxolane (235).

A solution of boronate **236** (32 mg, 0.14 mmol, 1.5 eq) in THF (5 ml) was treated with 1N aq. NaOH (1.1 ml, 5 eq) and the reaction mixture was stirred for 10 min at room temperature. Subsequently, $Pd(PPh_3)_4$ (10 mg, 0.009 mmol, 10 mol%) and vinyl chloride **237** (27 mg, 0.09 mmol, 1.0 eq) were added in sequence and the temperature was increased to 60 ^oC. The resulting mixture was allowed to stir for 2 h. Then, after having cooled, the reaction mixture was diluted with *n*-hexane (10 ml), dried ($MgSO_4$) and filtered through a short pad of silica gel. The filtrate was evaporated under reduced pressure and the crude product was purified by column chromatography (silica gel, hexane/EtOAc, 97:3) to yield compound **235** as a colorless oil (10 mg, 32%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.48 (d, J = 14.6 Hz, 1H), 5.81-5.25 (m, 3H), 4.82 (t, J = 4.8 Hz, 1H), 3.99-3.76 (m, 4H), 2.30 (ddt, J = 11.7, 7.6, 2.3 Hz, 2H), 1.69-1.26 (m, 12H), 1.14 (t, J = 7.5 Hz, 3H).^{13}C NMR (75 MHz, $CDCl_3$): δ 140.00, 125.80, 118.04, 113.65, 113.49, 105.05, 96.37, 95.23, 65.22, 34.27, 29.79, 29.41, 29.15, 29.03, 24.41, 20.07, 19.70, 14.21, 13.76. HRMS calcd. for $C_{20}H_{42}O_4$ $[M]^+$ 300.2089, found 300.2088.

Paper I

Synthesis of mycalazol and mycalazal analogs with potent antiproliferating activities.

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Synthesis of mycalazol and mycalazal analogs with potent antiproliferating activities*

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Abstract: Four 2,5-disubstituted pyrrole and two 2-disubstituted thiophene analogs of the natural products mycalazol 5 and mycalazal 2 have been prepared using the Stille coupling reaction. All four analogs displayed potent antiproliferating activity against several human cancer cell lines with IC_{50} values in the nanomolar range.

Keywords: antiproliferating activity; 2,5-disubstituted pyrroles; mycalazol 5; mycalazal 2; natural products; Stille coupling.

INTRODUCTION

Natural products are an important source for the development of new anticancer drugs [1]. Seldom is a natural product useful directly as a drug, but more often natural products are important as lead compounds for the development of a new anticancer drug. In those cases, structural–activity relationship (SAR) studies become necessary. However, before SAR studies can be performed, the synthesis of new analogs of the natural product of interest must be conducted [2]. Analogs are synthesized in order to enhance the biological activity, but also for achieving selectivity toward, for example, cancer cells. The new analogs should ideally exhibit improved pharmacokinetic as well as pharmacodynamic properties [2].

In 1997, Salvá and co-workers [3] reported the identification of 14 structurally related 2,5-disubstituted pyrroles from the northeastern Atlantic sponge *Mycale micracanthoxea*. The compounds exhibited interesting cytotoxic activity against several cancer cell lines. The presence of a saturated or an unsaturated carbon chain attached to the pyrrole ring characterizes these natural products. Recently, we reported the first total synthesis of mycalazol 5 (**1**) and mycalazal 2 (**2**) (Fig. 1) [4]. As part of our ongoing efforts on the synthesis and biological evaluation of polyunsaturated natural products [5] as potential new anticancer agents, we herein report the synthesis and antiproliferating activity of four new analogs of these naturally occurring 2,5-disubstituted pyrroles. The synthesis and biological evaluation of one 2-acyl thiophene analog and one 2-alkyl thiophene analog are also presented.

Recently, it was reported by Zhou, Nagle, and co-workers that 2,5-disubstituted pyrroles, such as **4** and **5**, isolated from a *Mycale* sponge inhibited hypoxia-inducible factor-1 (HIF-1α) in a human breast cancer cell assay [6]. Identification of new inhibitors of HIF-1α is an attractive approach for the development of new anticancer agents [7–9]. However, limited information on the SAR of analogs of the mycalazol and mycalazal class of natural products has so far been published. Nabbs and Abell reported the synthesis and cytotoxic properties data of three saturated analogs of mycalazol 11 (**3**) [10]. Hence,

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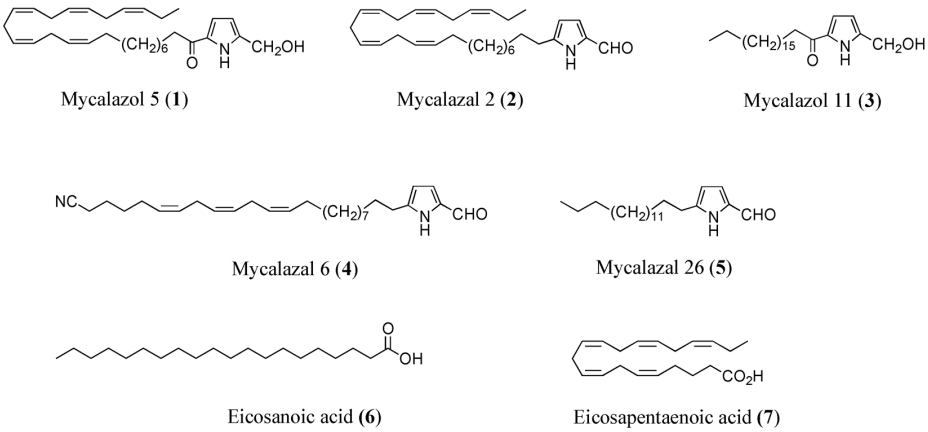


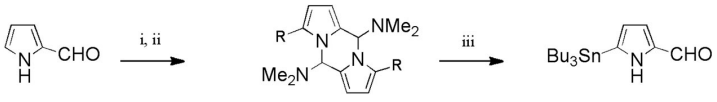
Fig. 1 Structures of mycalazol 5 (**1**), mycalazal 2 (**2**), mycalazol 11 (**3**), mycalazal 6 (**4**), mycalazal 26 (**5**), eicosanoic acid (**6**), and eicosapentaenoic acid (**7**).

based on our published synthesis of mycalazol 5 (**1**), we decided to prepare analogs with an unsaturated C-20 side chain and a saturated C-20 side chain at the 2-position of the pyrrole ring. For comparison, we also decided to prepare the 2-acyl substituted thiophene **18** and the 2-alkyl substituted thiophene derivative **19** with a saturated C-20 side chain. These six compounds, as well as the natural products **1** and **2**, were subjected to biological testing in four human cancer cell lines.

RESULTS AND DISCUSSION

Synthesis

The synthesis of analogs **13**, **14**, **15**, and **16** started with the preparation of 5-(*tri*-*n*-butylstannyl)pyrrole-2-carboxaldehyde (**10**) from commercial available pyrrole 2-carboxaldehyde **8** [11] via the known compound **9a** [12] (Scheme 1). First, an aqueous solution of dimethyl amine was reacted with pyrrole-2-carboxaldehyde (**8**) that yielded *aza*fulvene **9a** in a quantitative yield. Compound **9a** was then treated with 2.3 equiv of *n*-butyl lithium followed by reaction with excess *n*-tributyltin chloride. This afforded the known *aza*fulvene derivative **9b**. Then aqueous hydrolysis of **9b** afforded 5-(*tri*-*n*-butylstannyl)pyrrole-2-carboxaldehyde (**10**).

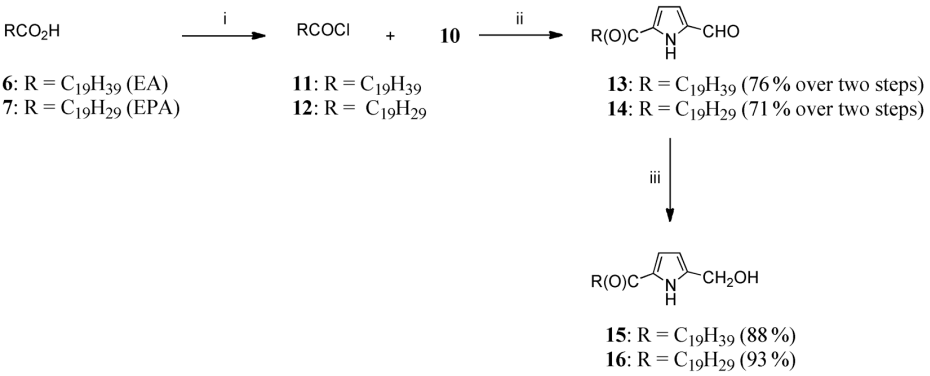


8 **R = H: 9a** **R = SnBu$_3$: 9b** **10**

i) $HNMe_2$, H_2O, 25 °C ii) a) *n*-BuLi, THF b) *n*-Bu_3SnCl, THF, -78 - 25 °C
iii) 0.4 M NaOAc, H_2O, THF, Δ (57 % from **8**).

Scheme 1 Synthesis of stannyl pyrrole 10.

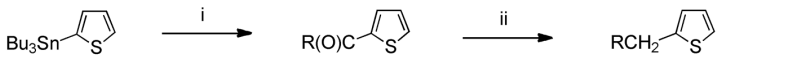
The commercially available eicosanoic acid (EA, **6**) and eicosapentaenoic acid (EPA, **7**) were converted into their respective acid chlorides **11** and **12** by reaction with oxalyl chloride in methylene chloride. The crude acid chlorides underwent Stille cross-coupling reaction [13] with the stannyl pyrrole **10** affording compounds **13** and **14** in 76 and 71 % yields, respectively (Scheme 2). Chemioselective reduction of the formyl group in **13** and **14** with $Zn(BH_4)_2$ [14] in THF at 0–25 °C afforded **15** and **16** in 88 and 93 % yields, respectively.



i) $(COCl)_2$, CH_2Cl_2 ii) $Pd(PPh_3)_4$, THF, Δ iii) $Zn(BH_4)_2$, THF, 0-25 °C.

Scheme 2 Synthesis of mycalazol 5 and mycalazal 11 analogs.

The 2-acyl thiophene analog **18** was prepared in 84 % yield from the commercially available 2-(*tri*-*n*-butylstannyl)pyrrole **17** and acid chloride **11** again using the Stille coupling. Reduction of the carbonyl group in **18** under standard hydrogenation conditions (Pd/C, H_2, EtOH) in the presence of catalytic amounts of sulfuric acid afforded thiophene **19** in 87 % yield (Scheme 3). Spectral data were in agreement with the assigned structures. The preparation of mycalazol 5 (**1**) and mycalazal 2 (**2**) has previously been described [4].



17 **18**: R = $C_{19}H_{39}$ (84 %) **19**: R = $C_{19}H_{39}$ (87 %)

i) **5a**, $Pd(PPh_3)_4$, THF, Δ ii) Pd/C, H_2, EtOH, H_2SO_4

Scheme 3 Synthesis of thiophene analogs **18** and **19**.

Biological results

Mycalazol 5 (**1**), mycalazal 2 (**2**) and the four pyrrole derivatives **13**, **14**, **15**, and **16** and the two thiophene analogs **18** and **19** were applied to four human cancer cell lines for the determination of their in vitro antiproliferating activity [15]. The results are summarized in Table 1. Mycalazol 5 (**1**) and mycalazal 2 (**2**) were the two most potent compounds in all four cell lines. In general, **1** was slightly

more active than **2** exhibiting IC_{50} values in the ranges 2.1–14.9 nM and 4.1–23.3 nM, respectively. This is in accord with the results reported by Salvá and co-workers [3]. Analogs **13** and **14** were less active than **1** and **2**. Interestingly, the presence of both an acyl-group and a formyl-group in the 2- and 5-positions does not enhance the antiproliferating activity. The IC_{50} values ranged from 11.3 to 22.9 nM for **14** and 13.0 to 31.1 nM for **13**. Only a slight increase in activity was observed in all cell lines with a polyunsaturated C-20 side chain in compound **16** compared to the saturated derivative **15**. Moreover, both compounds were less active than both mycalazol 5 (**1**) and mycalazal 2 (**2**). The IC_{50} values ranged from 27.7 to 38.5 nM for **15** and 20.5 to 34.7 nM for **16**. A further reduction was observed for the 2-acyl substituted thiophene **18** with IC_{50} values from 45.5 to 58.7 nM. The 2-alkyl thiophene **19** was inactive in all cancer cell assays.

Table 1 Antiproliferating activity data of mycalazol 5 (**1**), mycalazal 2 (**2**) and analogs.

Comp.	SKOV[a]	OVCAR[a]	WM35[a]	WM239[a]	VERO[a]
1	14.9 (±2.1)	9.4 (±1.8)	2.9 (±0.9)	2.1 (±0.9)	2.1 (±0.9)
2	23.3 (±3.0)	18.0 (±2.1)	4.1 (±1.0)	4.3 (±0.8)	4.3 (±0.8)
13	31.1 (±2.3)	24.1 (±1.5)	13.0 (±1.3)	15.9 (±1.9)	15.9 (±1.9)
14	22.9 (±1.9)	21.7 (±2.8)	11.3 (±1.9)	12.1 (±2.1)	12.1 (±2.1)
15	38.1 (±2.1)	38.5 (±2.2)	36.4 (±2.1)	27.7 (±2.2)	27.7 (±2.2)
16	34.3 (±3.3)	32.7 (±4.1)	24.1 (±2.6)	20.5 (±2.9)	20.5 (±2.9)
18	49.1 (±2.1)	58.7 (±3.3)	45.5 (±3.3)	58.0 (±2.9)	58.0 (±2.9)
19	>100	>100	>100	>100	>100

[a]Values (nM) are means of three experiments in each cell assay; standard deviation is given in parentheses.

The biological results reported herein indicated that the NH-pyrrole ring is important for the observed antiproliferating activity against all four human cancer cell lines. Moreover, both mycalazol 5 (**1**) and mycalazal 2 (**2**) as well as all new analogs except **19**, exhibited potent activity in the non-cancerous VERO cell line. These results, in combination with the results from the cancer cell lines, showed that the compounds exhibited low selectivity. Hence, other analogs of the mycalazols and mycalzals should be prepared with antiproliferating activity toward only the cancer cell lines.

CONCLUSION

The Stille cross-coupling reaction was employed for efficient syntheses of mycalazol and mycalazal analogs without protection of the NH-pyrrole. Moreover, the conservation of the all-*Z*-configuration of the methylene-interrupted double bonds was observed for natural products 1 and 2, as well as analogs **14** and **16**. In vitro antiproliferating activity against four human cancer cell lines was determined. All four 2,5-disubstiuted pyrrole analogs revealed significant activity in both ovarian and melanoma human cancer cell lines. The most active analog was **14**. For the thiophene analogs, only compound **18** exhibited antiproliferating activity. However, all analogs were less active than the natural products mycalazol 5 (**1**) and mycalazal 2 (**2**) and low selectivity was observed. Since inhibitors of HIF-1α continue to be of interest as potential remedies against various cancers, further structural–activity studies will focus on the synthesis and biological evaluation of additional analogs of mycalazol 5 (**1**). These efforts will be reported in due time.

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Supporting information

Synthesis of mycalazol and mycalazal analogs with potent antiproliferating activities

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Experimental

Melting points were uncorrected. Analytical TLC was performed on silica gel 60 F_{254} Aluminium sheets (Merck). Flash column chromatography ws performed on silica gel 60 (40-60 μm, Fluka). IR spectra (4000-600 cm^{-1}) were obtained on a Perkin-Elmer Spectrum BX series FT-IR spectrophotometer. NMR spectra were recorded on a Bruker Avance DPX 300 MHz spectrometer for ^{1}H-NMR and 75 MHz for ^{13}C-NMR. Coupling constants (*J*) are reported in Hertz, and chemical shifts are reported in ppm (δ) relative to $CDCl_3$. Mass spectra were recorded at 70 eV with Fission's VG pro mass spectrometer. High resolution mass spectra were performed with VG Prospec mass spectrometer.

Chemistry

Preparation of 5-(*tri-n*-butylstannyl)pyrrole-2-carboxyldehyde (10).

Pyrrole-2-carboxylaldehyde **8** (1.5 g, 15.79 mmol) was dissolved in aqueous dimethylamine (40 % wt, 20 ml) and the reaction mixture was allowed to stir for 3 h at room temperature. The obtained white precipitate was collected by filtration and washed with water and 1 M NaOH (10 ml), then recrystallized from EtOAc:Et_2O (1:1) to afford azafluvene dimer **9** as colorless crystals (2.3 g, 60%, EtOAc:Et_2O). M.p. 113 ^{o}C, literature 113^{o}C [12]. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.32 (s, 6H), 5.85 (s, 1H), 6.31 - 6.12 (m, 2H), 6.94 (dd, J = 2.7, 1.7 Hz, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 39.58 (2xCH_3), 72.43, 106.02, 108.82, 120.01, 125.83. The major steroisomer (96%) has the two NMe_2 group in *trans* relationship. To a stirred solution of azafulvene dimer **9** (1 g, 4.09 mmol) in THF (25 ml) at -15 ^{o}C, was *n*-BuLi (1.6 M in hexane, 5.87 ml, 9.40 mmol) added. The reaction mixture stirred for 15 min at -15 ^{o}C, then for 30 min at 0 ^{o}C and 1 h at room temperature. The deep violet solution was cooled at -78 ^{o}C and (1.9 mg, 5.87 mmol) of *n*-Bu_3SnCl was added slowly. The reaction mixture was stirred for 3 h at room temperature. The

reaction mixture was treated with aqueous AcONa (50 mL) under reflux for 3 days. After extraction (CH_2Cl_2, 20 mL) and evaporation of the solvent, the crude product was purified by flash chromatography (silica gel, EtOAc: *n*-Hexane 1:9) to yield **10** as a yellow oil (0.89 g, 57%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.91 (t, 3H, J = 7.3 Hz), 1.13-1.63 (m, 18H), 6.43 (dd, 1H, J = 2.3 and 3.7 Hz), 7.05 (dd, 1H, J = 2.3 and 3.7 Hz), 9.52 (s, 1H), 9.66 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 8.20 (3xCH_2), 14.00 (3xCH_3), 27.62 (3xCH_2), 30.71 (3xCH_2), 121.51, 121.91, 137.01, 137.01, 142.50, 178.44.

Preparation of eicosanoyl chloride (11)

Eicosanoic acid **6** (312 mg, 1 mmol) in dry CH_2Cl_2 (5 ml) was heated at 50 ^{o}C until all of the eicosanoic acid was dissolved. Oxalayl chloride (0.15 ml, 1.2 mmol) was added to the solution and the mixture was allowed to stir at 50 ^{o}C for 2 h. Evaporation of the solvent gave the acid chloride **11** as a white solid in quantitative yield that was used directly in the next step.

Preparation of (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-eicosa-5,8,11,14,17-pentaenoyl acid chloride (12)

Oxalyl chloride (0.15 g, 1.2 mmol) was added dropwise to a stirred solution of eicosapentaenoic acid **7** (302 mg, 1 mmol) in dicloromethane (5 ml) at room temperature for 2 h. The solvent was evaporated to obtain the eicosapentaenoic acid chloride **12** as yellow oil in quantitative yield that was used directly in the next step.

Synthesis of 5-eicosanoyl-1-pyrrole-2-carbaldehyde (13):

The eicosanoic acid chloride **11** (170 mg, 0.50 mmol) was dissolved in dry THF (25 ml) followed by addition of stannyl pyrrole **10** (190 mg, 0.50 mmol. Palladium tetrakis (7.5 mg, 0.05 mmol) was added and the mixture was stirred at reflux under argon for 6 hrs. The reaction was quenched with addition of water (50 ml) and extracted with Et_2O (3x50 ml). The combined organic layer was washed with brine and dried ($MgSO_4$). The solvent was evaporated and the crude product was purified by flash chromatography (silica gel, EtOAc:*n*-hexane 1:9) as eluent to yield **13** as a white solid (0.14 g, 76%). R_f= 0.48 (*n*-hexane:EtOAc 1:9); m.p. 92-93 ^{o}C; IR (film): 3368, 2945, 1681, 1654 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.85 (t, 3H, J = 6.4 Hz), 1.23-1.30 (m, 32H),

1.64-1.70 (m, 2H), 1.94 (t, 2H, *J* = 7.6 Hz), 6.87 (dd, 1H, *J* = 2.6 and 4.0 Hz), 6.93 (dd, 1H, *J* = 2.6 and 4.0 Hz), 9.68 (s, 1H), 9.98 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.51, 23.08, 24.93, 29.69, 29.75, 29.78, 29.85, 29.99, 30.06, 30.09 (8 x CH_2), 32.32, 39.20, 115.60, 119.96, 135.01, 135.84, 181.12, 192.65; MS EI: m/z = 389 (M^+); HRMS calcd for $C_{25}H_{43}NO_2$ (M^+): 389.3299, found 389.2938.

Synthesis of 5-(5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-eicosa-5,8,11,14,17-pentaenoyl-pyrrole-2-carbaldehyde (14): The acid chloride **12** (160 mg, 0.50 mmol) was dissolved in dry THF (25 ml, ca 0.5 M) followed by addition of stannyl pyrrole **10** (190 mg, 0.50 mmol. Palladium tetrakis (7.5 mg, 0.05 mmol) was added and the mixture was stirred at reflux under argon for 6 hrs. The reaction was quenched with addition of water (50 ml) and extracted with Et_2O (3x50 ml). The combined organic layer was washed with brine and dried ($MgSO_4$). The solvent was evaporated and the crude product was purified by flash chromatography (silica gel, EtOAc:*n*-hexane 1:9) as eluent to yield **14** as a yellow oil; R_f = 0.40 (*n*-hexane:EtOAc 1:9); IR (film): 3435, 3054, 2987, 1681, 1657 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.94 (t, 3H, *J* = 7.5), 1.75-1.84 (m, 2H), 2.00-2.17 (m, 4H), 2.76-2.85 (m, 10H), 5.24-5.36 (m, 10H), 6.86 (dd, 1H, *J* = 2.6 and 4.0), 6.92 (dd, 1H, *J* = 2.6 and 4.0), 9.68 (s, 1H), 10.00 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.66, 20.95, 24.53, 25.94, 26.04, 26.05, 26.98, 30.77, 38.40, 115.68, 119.93, 127.04, 128.25, 128.45, 128.54, 128.57, 128.68, 128.99, 129.38, 129.43, 132.45, 135.09, 135.80, 181.18, 192.29; MS EI: (m/z) = 379 (M^+); HRMS calcd for $C_{25}H_{33}NO_2$ (M^+): 379.2505, found 379.2511.

Synthesis of 1-(5-(hydroxymethyl)-1-pyrrol-2-yl)-eicosan-1-one (15).

$ZnCl_2$ (3.4 g, 25.56 mmol) was added to sodium boronhydride (1.95 g, 52.70 mmol) in THF (50 ml). The mixture was stirred overnight at 0-5 ^{o}C. After filtration, the resulting clear solution was used immediately. A freshly prepared solution of $Zn(BH_4)_2$ (2 ml, ca 0.5 M) was added drop wise over 5 min to a solution of **13** (60 mg, 0.15 mmol) in THF (5 ml) at room temperature. After 1 h of stirring, the reaction mixture was triturated with saturated NH_4Cl until no more gas evolved. The mixture was extracted with ether. The combined organic phase was washed with saturated $NaHCO_3$ (20 ml) and dried ($MgSO_4$). After evaporation of the solvent the crude product was purified by flash chromatography (silica gel, EtOAc:*n*-hexane, 1:1) to yield **15** as a white solid (53 mg, 88%). R_f= 0.10 (*n*-hexane:EtOAc 1:1); m.p. 94-95 ^{o}C; IR (film): 3368 (br), 2944, 1654,

1622 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 0.86 (t, 3H, J = 7.6 Hz), 1.27-1.35 (m, 32H), 1.67-1.74 (m, 2H), 2.79 (t, 2H, J = 7.6 Hz), 3.64 (br s, 1H), 4.71 (s, 2H), 6.12 (dd, 1H, J = 2.6 and 3.6 Hz), 6.89 (dd, 1H, J = 2.6 and 3.6 Hz), 10.73 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.51, 23.09, 26.04, 29.76, 29.81, 29.82, 29.90, 30.02, 30.05, 30.10 (8 x CH_2), 32.32, 38.23, 58.25, 109.09, 118.51, 132.00, 140.76, 192.58; MS EI: (m/z) = 391 (M^+); HRMS calcd for $C_{25}H_{45}NO_2$ (M^+): 391.3463, found 391.3450.

Synthesis of (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-1-(5-(hydroxymethyl)-1-pyrrol-2-yl)eicosa-5,8,11,14,17-pentaen-1-one (16): A freshly prepared solution of $Zn(BH_4)_2$ (2ml) was added drop wise over 5 min to a solution of **14** (60 mg, 0.16 mmol) in THF (5 ml) at room temperature. After 1 h of stirring, the reaction mixture was triturated with saturated NH_4Cl until no more gas evolved. The mixture was extracted with ether. The combined organic phase was washed with saturated $NaHCO_3$ (x ml) and dried ($MgSO_4$). After evaporation of the solvent the crude product was purified by flash chromatography (silica gel, EtOAc:*n*-hexane, 1:1) to yield **16** as a pale yellow oil (56.5 mg, 93%); R_f = 0.12 (*n*-hexane:EtOAc 1:1); IR (film): 3436, 3055, 2988, 1652, 1629 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 0.95 (t, 3H, J = 7.5 Hz), 1.73-1.83 (m, 2H), 2.00-2.17 (m. 4H), 2.74-2.82 (m, 10H), 3.15 (br t, 1H), 4.71 (d, 2H, J = 6.0 Hz), 5.25-5.39 (m, 10H), 6.13 (dd, 1H, J = 2.6 and 3.7 Hz), 6.87 (dd, 1H, J = 2.6 and 3.7 Hz), 10.39 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.51, 20.95, 23.05, 25.57, 25.94, 26.03, 27.19, 31.98, 37.54, 58.31, 108.99, 118.04, 127.41, 128.28, 128.51 (2 x CH), 128.66 (2 x CH), 128.98, 129.22, 129.60, 132.01, 132.46, 139.93, 191.74; MS EI: (m/z) = 381 (M^+); HRMS calcd for $C_{25}H_{35}NO_2$ (M^+): 381.2670, found 381.2667.

Synthesis of 1-(thiophen-2-yl)eicosan-1-one (18): The eicosanoic acid chloride **11** (170 mg, 0.50 mmol) was dissolved in THF (25 ml) and added to a solution of stannyl thiophene **16** (187 mg, 0.50 mmol). Then the reaction mixture was refluxed under argon for 6 hrs. The reaction monitored by TLC. The reaction was quenched by addition of water (50 ml), and extracted by Et_2O (3x50 ml). The combined organic layer was washed by brine then dried ($MgSO_4$). The solvent was evaporated and the crude products were purified by flash chromatography using eluent system (*n*-Hexane) to yield **16** as yellow oil (84 %); IR (film): 1724, 1622 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 0.85 (t, 3H, J = 7.4 Hz), 1.18-1.38 (m, 28H), 1.70-1.74 (m, 4H), 2.74 (m,

2H), 3.34 (t, 2H, J = 7.5 Hz), 7.13 (dd, 1H, J = 3.5 and 4.0 Hz), 7.61 (d, 1H, J = 1.5 and 4.0 Hz), 7.72 (dd, 1H, J = 1.5 and 3.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.65, 23.69, 25.91, 26.02, 26.11, 27.19, 28.11, 28.91, 29.21, 29.51 (8 x CH_2), 32.02, 37.22, 128.41, 131.28, 133.66, 144.80, 193.1; HRMS calcd for $C_{24}H_{42}SO$ (M^+): 378.2956, found 378.2929.

Synthesis of 2-eicosylthiophene (19): 1-(Thiophen-2-yl)eicosan-1-one **17** was dissolved in EtOH followed by addition of Pd/C (10.6 mg, 0.1 mmol) and H_2SO_4 (0.1 ml, 3.3M). The reaction was allowed to stir for 14 hrs and then filtered through a plug of silica gel. Evaporation of the solvent afforded a yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (t, 3H, J = 7.4 Hz), 1.20-1.32 (m, 32H), 1.70-1.74 (m, 4H), 2.80 (t, 2H, J = 7.4 Hz), 6.78 (dd, 1H, J = 3.6 and 4.0 Hz), 6.89 (d, 1H, J = 1.5 and 4.0 Hz), 7.19 (dd, 1H, J = 1.5 and 4.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.60, 22.99, 26.01, 26.16, 26.41, 27.80, 28.61, 28.82, 29.02, 29.25 (8 x CH_2), 32.32, 38.59, 41.51, 128.21, 131.88, 133.16, 144.57; HRMS calcd for $C_{24}H_{44}S$ (M^+): 364.3164, found 364.3149.

Bioassay

The method applied was a modified procedure described by Lawrence and coworkers.[9] The human melanoma (WM35 and WM239) and ovarian (SKOV, OVCAR) cancer cell lines were cultivated in RPMI 1640 medium (BioWhittaker Europe, Verviers, Belgium) containing 5 % (melanoma) and 10% FCS (PAA laboratories, Pasching, Austria) and 2 mM L-glutamine (BioWhittaker Europe, Verviers, Belgium). The WM35 and WM239 cell lines were kindly provided by Dr. Meenhard Herlyn (Wistar Institute, Philadelphia, PA, USA) whereas the ovarian cell lines were obtained from American Type Culture Collection (ATCC) (Manassas, VA, USA). 5000 cells in 100 µl regular growth medium were plated per well in 96 well plates and left to attach overnight. Thereafter, a dilution series of compound (in 100 µl) was added. Proliferation was measured after 72 h following labeling of the cells with 1 µCi [^{3}H]Thymidine (American Radiolabeled Chemicals, Inc, St. Louis, MO) for the last 24 h before harvesting using a Filtermate harvester (Packard Instrument Company, Meriden, CT). [^{3}H]Thymidine incorporation was assessed in a Packard Microplate Scintillation Counter (Packard Instrument Company). Controls were incubated with medium containing DMSO only.

Paper II

Polyunsaturated fatty acid-derived chromones exhibiting potent antioxidant activity.

Mohamed, Y. M. A.; Vik, A.; Hofer, T.; Andersen, J. H.; Hansen, T. V. *Chem. Phs. Lipids*, **2013**, submitted.

Paper III

First total synthesis of methyl (5*Z*,8*Z*,10*E*,12*E*,14*Z*)-eicosapentaenoate.

Mohamed Y. M. A.; Hansen T. V. *Tetrahedron Lett*. **2011**, *52*, 1057-1059.

Supporting information

Synthesis of Methyl (5*Z*,8*Z*,10*E*,12*E*, 14*Z*)-eicosapentaenoate

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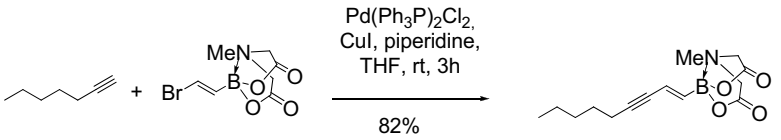
All reagents and solvents were used as purchased without further purification. Melting points were determined in open capillary tubes and were uncorrected. Analytical TLC was performed on silica gel 60 Merck. IR spectra (4000 - 600 cm^{-1}) were obtained on a Perkin-Elmer Spectrum BX series FT-IR spectrophotometer. NMR spectra were recorded on a Bruker Avance DPX - 300 MHZ spectrometer for ^{1}H NMR and 75 MHZ for ^{13}C NMR. Coupling constants (*J*) are reported in Hertz, and chemical shifts are reported in ppm (δ) relative to $CDCl_3$ (7.24 ppm for ^{1}H and 77.40 ppm for ^{13}C). Mass spectra were recorded at 70 eV with Fission's VG pro. High resolution mass spectra were performed with VG Prospec mass spectrometer.

Procedures and spectral data

(*E*)- Non-1-en-3-ynyl boronic acid mida ester (3).

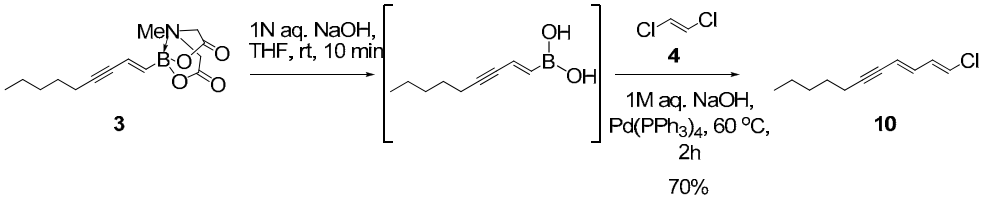


6 **7** **3**

To a mixture of (*E*)-2-bromovinylboronic acid mida ester **7** (262 mg, 1.00 mmol), $Pd(Ph_3P)Cl_2$ (35 mg, 5 mol%) and CuI (19 mg, 10 mol%) under Argon, dry THF (5 ml), piperidine (0.19 ml, 2.00 mmol) and 1-heptyne (**6**, 0.14 ml, 1.10 mmol) were added. The reaction mixture was stirred for 3 h at room temperature. The resulting mixture was diluted with EtOAc (10 ml) and then filtered through a short pad of silica gel using EtOAc as eluent. The filtrate was evaporated under reduced pressure and the residue was purified by flash chromatography using silica gel (Hexane/EtOAc, 1:1, Et_2O, Et_2O/EtOAc, 1:1, Et_2O/EtOAc, 3:7) to afford the title product as a colorless solid (0.23 g, 82%) m.p. 110-111 ^{o}C. IR (film): ν = 3054, 2986, 2125, 1770, 1602, 1550; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.11 -

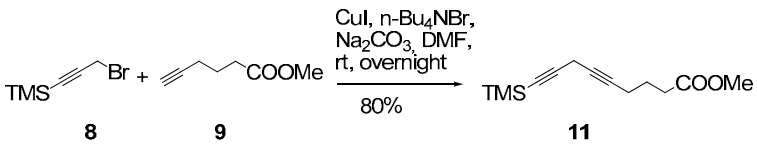
5.86 (m, 2H), 4.05 (d, *J*=17.0 Hz, 2H), 3.70 (d, *J*=17.0 Hz, 2H), 2.81 (s, 3H), 2.26 (td, *J*=7.0, 1.2 Hz, 2H), 1.54 - 1.23 (m, 1H), 0.86 (t, *J*=7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 168.98 (2 x CO), 124.88 (2 x CH), 93.24 (C≡C), 80.85 (C≡C), 61.98 (2 x CH_2), 47.57 (CH_3), 31.51 (CH_2), 28.75 (CH_2), 22.58 (CH_2), 19.81 (CH_2), 14.36 (CH_3). MS (EI) m/z: 277 $[M]^+$.

(1*E*,3*E*)-1-Chloroundeca-1,3-dien-5-yne (10).



To a solution of **3** (60 mg, 0.22 mmol, 1 eq) in THF (5 ml), 1M NaOH (aq.) (1.1 ml, 5 eq) was added. The reaction mixture was stirred for 10 min at room temperature. Then $Pd(PPh_3)_4$ (24.2 mg, 0.021 mmol, 10 mol%) and *trans*-1,2-dichloroethene (**4**, 52 mg, 0.54 mmol, 2.5 eq) were added. The temperature was increased to 60 °C and then the resulting mixture was allowed to stir for 2 h. The reaction mixture was cooled, diluted with *n*-hexane (10 ml) and dried ($MgSO_4$). The reaction mixture was filtered through a short pad of silica gel and the filtrate was evaporated. The residue was purified by flash chromatography on silica gel (n-hexane) to afford the title product **10** as colorless oil (27.6 mg, 70%). IR (film): ν = 3054, 2987, 2126, 1604, 1551; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.53 - 6.16 (m, 3H), 5.68 - 5.52 (m, 1H), 2.30 (td, *J*=7.1, 2.3 Hz, 2H), 1.60 - 1.14 (m, 6H), 0.88 (t, *J*=7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 136.27 (CH), 133.49 (CH), 122.36 (CH), 113.51 (CH), 94.96 (C≡C), 80.09 (C≡C), 31.48 (CH_2), 28.75 (CH_2), 22.59 (CH_2), 20.00 (CH_2), 14.35 (CH_3). MS (EI) m/z: 182 $[M]^+$.

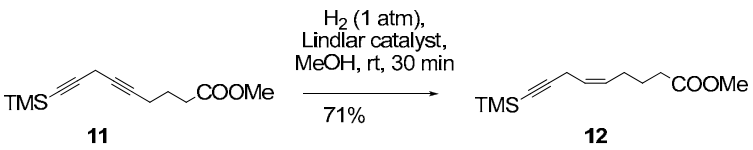
Methyl 9-(trimethylsilyl)nona-5,8-diynoate (11).[1]



The title compound was according to a literature procedure.[1] To a mixture of Na_2CO_3 (1.66 g, 15.7 mmol,1.5 eq), CuI (1.98 g, 10.5 mmol, 1eq), n-Bu_4NBr (1.01 g, 3.1 mmol, 0.3 eq) in DMF (12 ml) at -20 °C, 5-methyl hexynoate (**9**,1.32 g, 10.5 mmol, 1eq) was added, followed by addition of 3-trimethylsilyl propagyl bromide (2.38 g, 12.5 mmol, 1.2 eq). The reaction was allowed to stir at room temperature overnight. Et_2O (5 ml) was added, and filtered through a short pad of silica gel. Water (10 ml) was added and the mixture was extracted with Et_2O (3 x 20 ml). The

organic layer was washed with saturated NH_4Cl and dried ($MgSO_4$). The solvent was evaporated and the residue was purified by flash chromatography (silica gel, hexane/EtOAc, 95:5) to afford the title compound (1.97 g, 80%) as a colorless oil which soon turned to yellow. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.66 (s, 3H), 3.16 (t, J=2.4 Hz, 2H), 2.42 (t, J=7.4 Hz, 2H), 2.31 - 2.13 (m, 2H), 1.82 (q, J=7.4 Hz, 2H), 0.14 (s, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 174.04 (CO_2Me), 100.88 (C≡C), 85.22 (C≡C), 80.06 (C≡C), 74.83 (C≡C), 51.93 (CH_2), 33.20 (CH_2), 24.21 (CH_2), 18.60 (CH_2), 11.25 (CH_3), 0.29 (3 x CH_3).

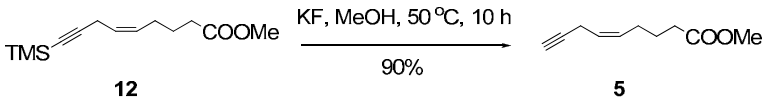
(*Z*)-Methyl 9-(trimethylsilyl)non-5-en-8-ynoate (12).[1]



11 **12**

The title compound was prepared by using a modified literature method.[1] To Lindlar catalyst ($Pd/CaCO_3$, 0.5 g, 50%wt), MeOH (10 ml) was added and the mixture was allowed to stir under Argon at room temperature. Then the Argon was replaced by Hydrogen. After saturation of the catalyst, quinoline (0.54 g, 4.20 mmol, 1 eq) was added followed by addition of the diyne **11** (1.00 g, 4.20 mmol, 1eq). After 30 min. the reaction mixture was filtered through a short pad of silica gel. The solvent was evaporated and the crude was purified by flash chromatography on silica gel (hexane:EtOAc, 97:3) furnishing **12** as a colorless oil (0.71 g, 71%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 5.53 - 5.34 (m, 2H), 3.64 (s, 3H), 2.93 (d, J=5.4 Hz, 2H), 2.29 (t, J=7.5 Hz, 2H), 2.16 - 1.98 (m, 2H), 1.70 (q, J=7.5 Hz , 2H), 0.11 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.38 (CO_2Me), 130.92 (CH), 125.43 (CH), 105.50 (C≡C), 84.67 (C≡C), 51.89 (OCH_3), 33.78 (CH_2), 26.88 (CH_2), 24.86 (CH_2), 18.74 (CH_2), 0.46 (3 x CH_3).

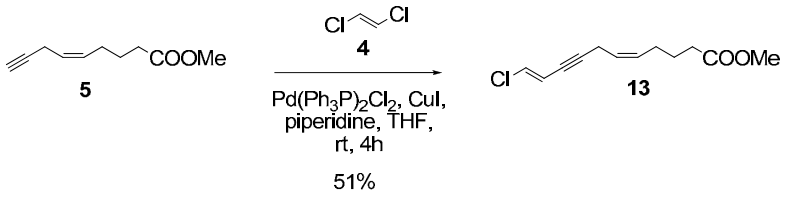
(*Z*)-Methyl non-5-en-8-ynoate (5).[1]



12 **5**

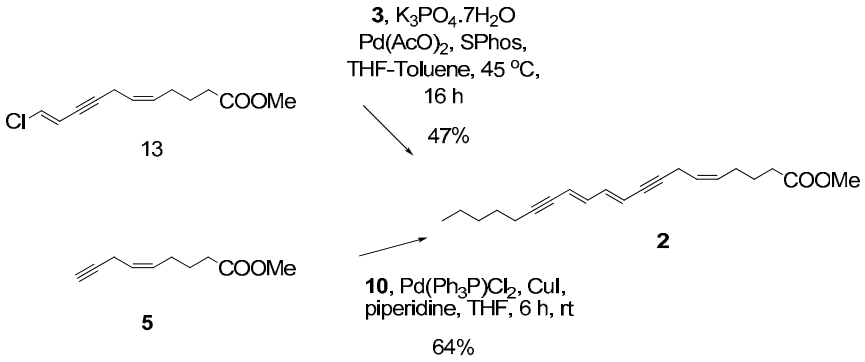
Compound **5** was prepared by a modified literature method. [1] To a solution of **12** (0.64 g, 2.7 mmol) in MeOH (10 ml), KF (1.56 g , 26.9 mmol) was added. The mixture was stirred for 10 h at 50 °C. After cooling the reaction mixture, it was diluted with water (25 ml) and extracted with Et_2O (3 x 20 ml). The organic layer was washed with brine and dried ($MgSO_4$). The solvent was evaporated and the crude product was purified by flash chromatography on silica gel (hexane:EtOAc, 98:2) to afford the title compound as a colorless oil (0.40 g, 90%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.56 - 5.32 (m, 2H), 3.65 (s, 3H), 2.91 (dd, J = 5.3, 2.7 Hz, 2H), 2.30 (t, J = 7.5 Hz, 2H), 2.17 - 2.01 (m, 2H), 1.95 (t, J = 2.7 Hz, 1H), 1.70 (q, J = 7.5 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.30 (CO_2Me), 131.19 (CH), 125.12 (CH), 83.02 (C≡C), 68.45 (C≡C), 51.90 (CH_3), 33.73 (CH_2), 26.82 (CH_2), 24.84 (CH_2), 17.22 (CH_2).

(5*Z*,*10E*)-Methyl 11-chloroundeca-5,10-dien-8-ynoate (13).



To a mixture of $Pd(Ph_3P)Cl_2$ (2.5 mg, 0.0036 mmol, 1 mol%) and CuI (6.8 mg, 0.036 mmol, 10 mol%) in dry THF (5 ml) under Argon, *trans* 1,2-dichloroethene (**4**) (0.17 ml, 1.80 mmol, 5 eq) and piperidine (0.10 ml, 1.08 mmol, 3 eq) were added. Then (*Z*)-methyl non-5-en-8-ynoate (**5**) (60 mg, 0.36 mmol, 1 eq) in THF (1 ml) was added. The reaction mixture allowed to stir for 4 h at room temperature. The resulting mixture was diluted with EtOAc (10 ml) and then filtered through short a pad of silica gel using EtOAc as eluent. The filtrate was evaporated under reduced pressure and the resulting crude product was purified by flash chromatography on silica gel (hexane/EtOAc, 95:5) to afford the title compound **13** as a colorless oil (42 mg, 51%). IR (film): ν = 3054, 2987, 2126, 1733, 1604; ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.44 (dd, *J* = 13.6, 6.0 Hz, 1H), 5.88 (dt, *J* = 13.6, 2.4 Hz, 1H), 5.53 - 5.33 (m, 2H), 3.65 (s, 3H), 3.01 (dd, *J* = 2.7, 1.6 Hz, 2H), 2.30 (t, *J* = 7.5 Hz, 2H), 2.07 (m, 2H), 1.70 (q, 7.5 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.29 (CO_2Me), 131.27 (CH), 129.60 (CH), 124.85 (CH), 114.45 (CH), 91.46 (C≡C), 75.89 (C≡C), 51.90 (OCH_3), 33.71 (CH_2), 26.85 (CH_2), 24.82 (CH_2), 18.17 (CH_2); MS (EI) m/z: 226 $[M]^+$.

(5*Z*,10*E*,12*E*)-Methyl eicosa-5,10,12-trien-8,14-diynoate (2).

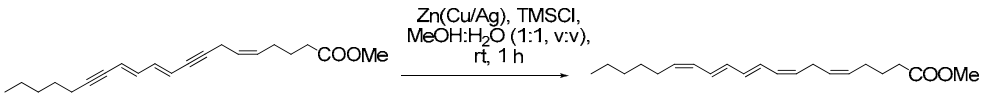


Procedure 1: Suzuki-Miyaura reaction. To a solution of **3** (68 mg, 0.26 mmol, 1.5 eq) in THF (3 ml), 1 M aq. NaOH (0.52 ml, 3 eq) was added. The reaction mixture was allowed to stir for 15 min at room temperature then the reaction was quenched with saturated NH_4Cl (2 ml), diluted with Et_2O, extracted with (THF/Et_2O, 1:1, 3 x 3 ml). The solvent was reduced to remaining ca 2 ml. A solution of $Pd(AcO)_2$ (0.0083 M, 5.6 mg, 0.025 mmol) and SPhos

(20.5 mg, 0.05 mmol) in toluene (3 ml) was allowed to stir under Argon at 65 ^{o}C for 15 min. To a mixture of **13** (38.4 mg, 0.17 mmol, 1 eq) and K_3PO_4•$7H_2O$ (0.172 g, 0.51 mmol, 3 eq) in THF (2 ml), the bronic acid (2 ml) and a solution of Pd catalyst (0.4 ml, 2 mol% Pd) were added sequentially. The reaction mixture was allowed to stir for 16 h at 45 ^{o}C. The mixture was cooled then diluted with EtOAc (5 ml) then filtered through short pad of silica gel. The solvent was evaporated to give the crude product that was purified by flash chromatography on silica gel (hexane/EtOAc, 98:2) to afford the compound (25 mg, 47%). IR (film): ν = 3054, 2986, 2125, 1770, 1602; ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.58 - 6.39 (m, 2H), 5.68 - 5.34 (m, 4H), 3.65 (s, 3H), 3.05 (d, J = 3.7 Hz, 2H), 2.30 (t, J = 7.4 Hz, 4H), 2.09 (q, J = 7.5 Hz, 2H), 1.71 (q, J = 7.5 Hz, 2H), 1.50 (p, J = 7.4 Hz, 2H), 1.34 - 1.20 (m, 4H), 0.88 (t, J = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.32 (CO_2Me), 140.41 (CH), 139.90 (CH), 131.01 (CH), 125.27 (CH), 113.91 (CH), 113.24 (C≡C), 95.45 (C≡C), 92.68 (C≡C), 80.06 (C≡C), 51.90 (OCH_3), 33.74 (CH_2), 31.48 (CH_2), 28.79 (CH_2), 26.85 (CH_2), 24.87 (CH_2), 22.60 (CH_2), 20.06 (CH_2), 18.47 (CH_2), 14.35 (CH_3); UV, λ_{max} (hexane, nm) = 294 (62500), 308 (65400). MS (EI) m/z: 312 $[M]^{+}$.

Procedure 2: Sonogashira cross coupling reaction. To a mixture of $Pd(Ph_3P)Cl_2$ (12 mg, 0.017 mmol, 5 mol%) and CuI (6.5 mg, 0.034 mmol, 10 mol%) in THF (5 ml) under Argon, piperidine (0.10 ml, 1.05 mmol, 3 eq) and (1*E*,3*E*)-1-chloroundeca-1,3-dien-5-yne **10** (58 mg, 0.32 mmol, 1 eq) were added, followed by the addition of (*Z*)-methyl non-5-en-8-ynoate **5** (58 mg, 0.35 mmol, 1.1 eq) in THF (1 ml). The reaction mixture was allowed to stir for 6 h at room temperature. The resulting mixture was diluted with EtOAc (10 ml) then filtered through a short pad of silica gel using EtOAc as eluent. The solution was washed with saturated NH_4Cl, dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel (hexane/EtOAc, 98:2) to afford the title product **2** as a colorless oil (70 mg, 64%).

Methyl (5*Z*,8*Z*,10*E*,12*E*, 14*Z*)-eicosapentaenoate (1b).



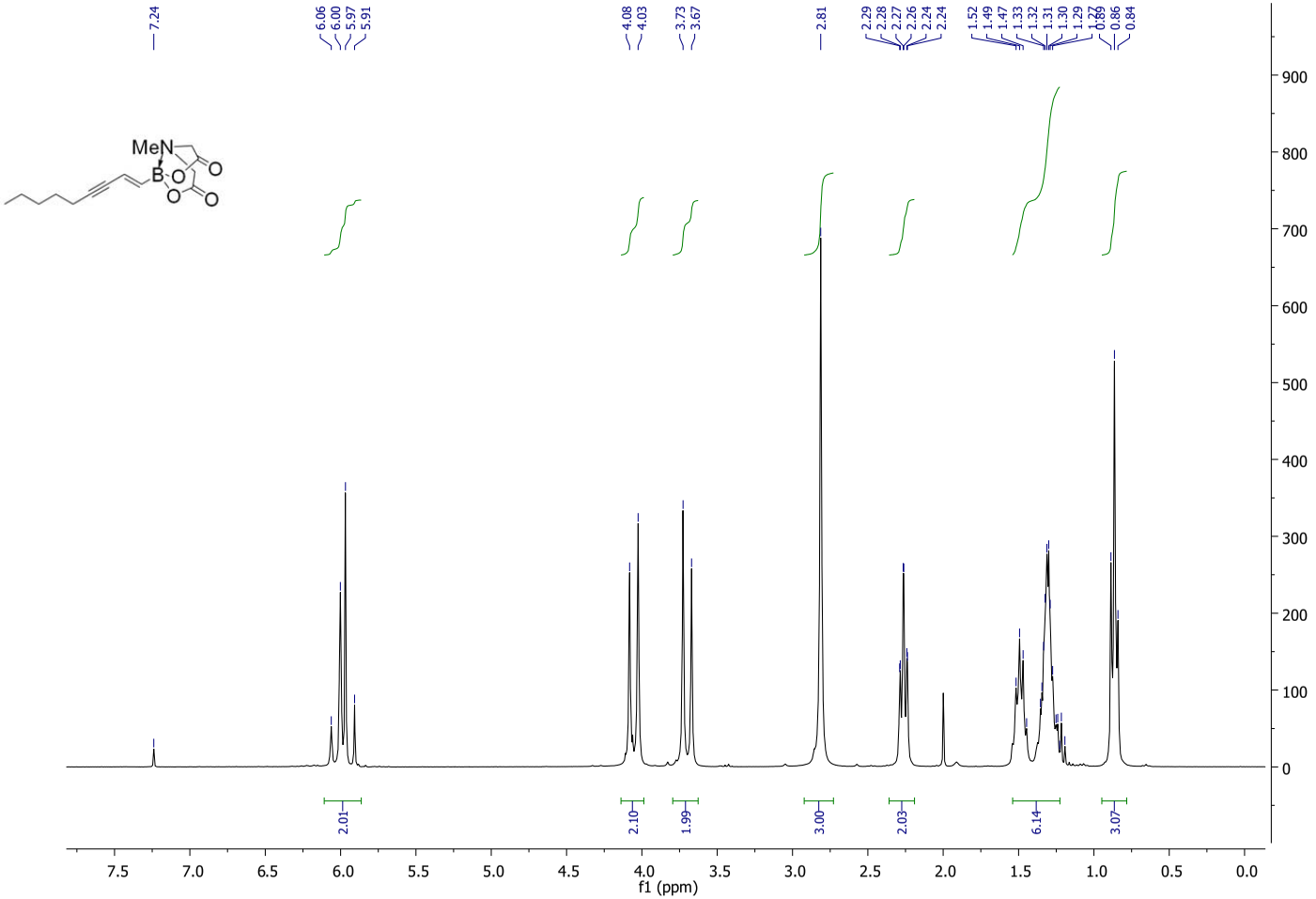
2 **1b**

To a suspension of Zn(Cu/Ag) (2.5 g) prepared according to a literature method[2] in MeOH/H_2O (6 ml, 1:1, v:v) followed by addition of the diyne **2** (24 mg, 0.08 mmol) dissolved in 1 ml MeOH and TMSCl (0.1 ml, 1.08 mmol) was added. The reaction was allowed to stir at room temperature for 1 h. The reaction mixture was filtered through a short pad of silica gel by eluting with MeOH. The solvent was concentrated to (1/3) of its original volume and a mixture of hexane/EtOAc, (1:1) (20 ml) was added. The organic layer was washed with brine (10 ml), dried ($MgSO_4$) and evaporated to give a crude product that was purified by flash chromatography on silica gel (hexane/EtOAc, 98.5:1.5) to afford **1b** as a colorless oil (21 mg, 88 %). R_f = 0.34 (EtOAc/hexane, 1:9); IR (film): ν = 3054, 2987, 1733,1601; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.47 (dd, J = 13.4, 10.8 Hz, 2H), 6.32 - 6.22 (m, 2H), 6.10 - 5.95 (m, 2H), 5.52 - 5.26 (m, 4H), 3.65 (s, 3H), 2.91 (t, J = 6.2 Hz, 2H), 2.31 (t, J = 7.5 Hz, 2H), 2.23 - 2.03 (m,

4H), 1.69 (p, J = 7.4 Hz, 2H), 1.45 - 1.17 (m, 6H), 0.87 (t, J = 6.8 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.40 (CO_2Me), 133.81 (CH), 133.68 (CH), 132.99 (CH), 130.47 (CH), 129.67 (CH), 129.34 (CH), 129.03 (CH), 128.97 (CH), 128.79 (CH), 128.04 (CH), 51.87 (OCH_3), 33.81 (CH_2), 31.87 (CH_2), 29.73 (CH_2), 28.32 (CH_2), 26.97 (CH_2), 26.61 (CH_2), 25.14 (CH_2), 22.93 (CH_2), 14.43 (CH_2); UV λ_{max} (hexane, nm): 294 (e = 56250), 305 (e = 57200), 320 (e = 47800); (literature,[3] λ_{max} (hexane) = 293, 306, 321). MS (EI) m/z: 317 $[M+1]^+$ (9), 316 $[M]^+$ (55), 119 (32), 106 (18), 91 (100), 79 (64), 67 (80), 55 (60). HRMS (EI) $C_{21}H_{32}O_2$ requires 316.2402, found 316.2412.

References

1. Chemin, D.; Gueugnot S.; Linstrumelle G. *Tetrahedron* **1992**, 48, 4369-4378.
2. Boland, W.; Schroer, N.; Sieler C.; Feigel, M. *Helvetica Chem. Acta* **1987**, 70, 1025-1040
3. Mikhailova M. V.; Bemis D. L.; Wise M. L.; Gerwick W. H.; Norris J. N.; Jacobs R. S. *Lipids* **1995**, 30, 583–589.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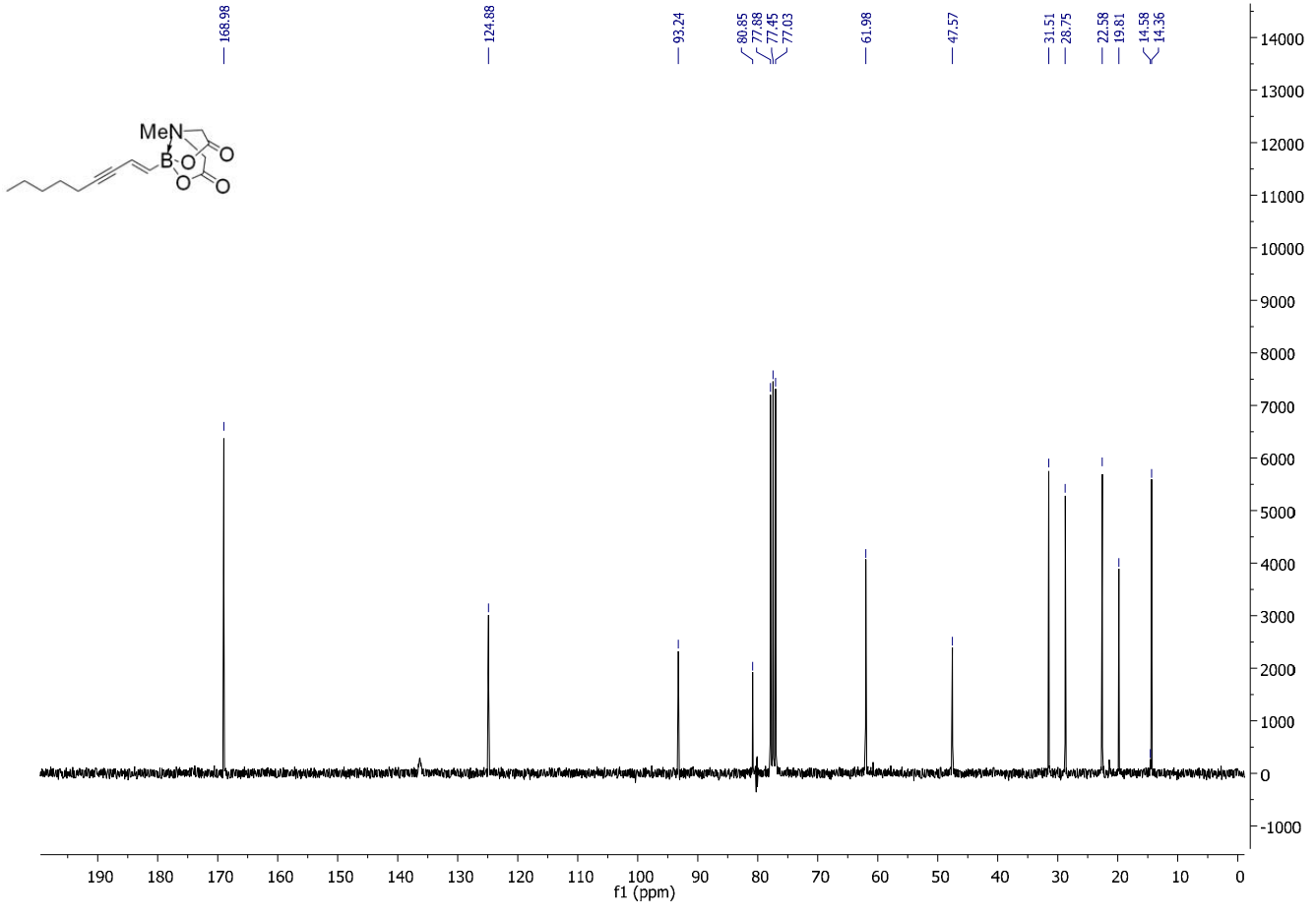
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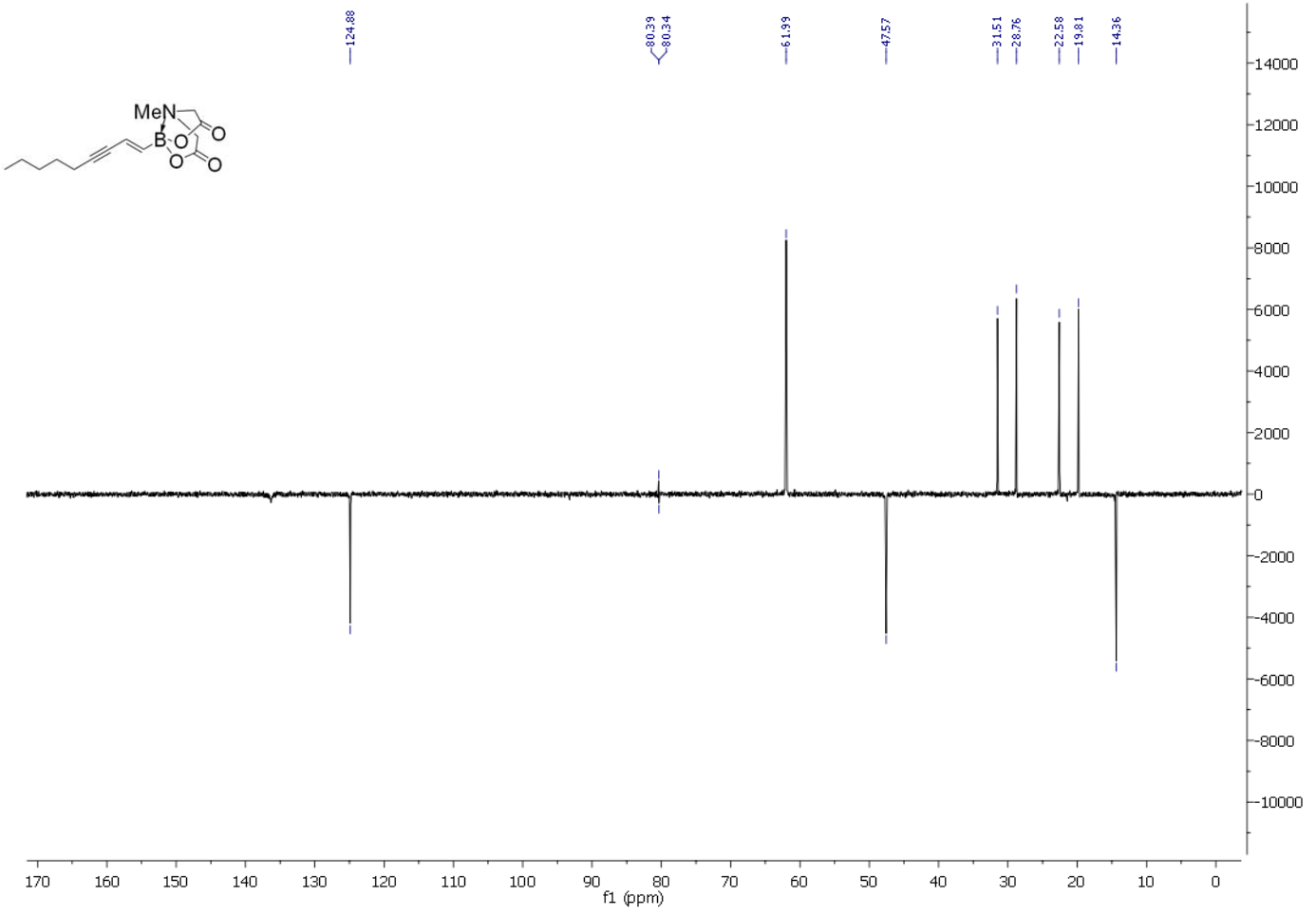
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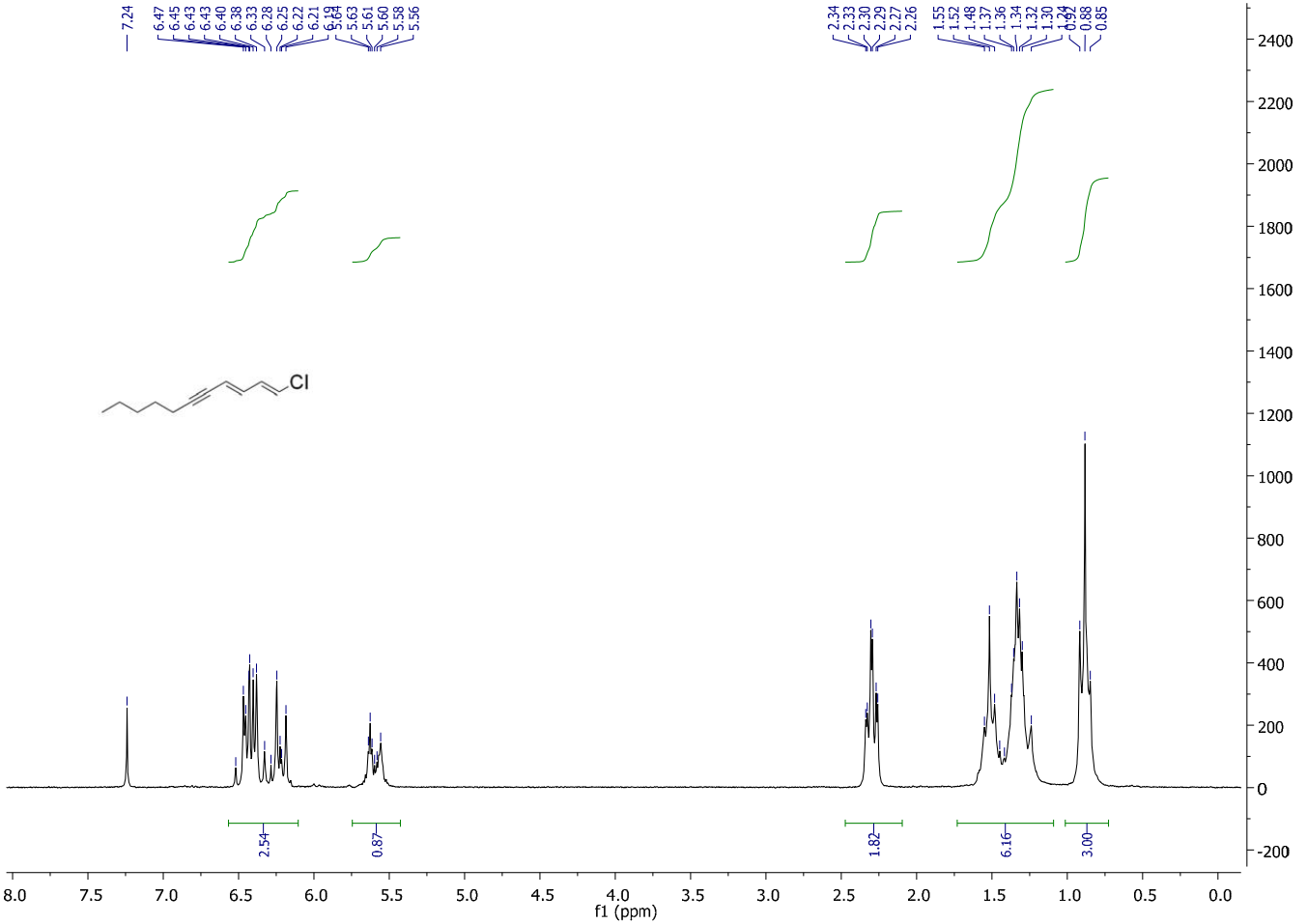
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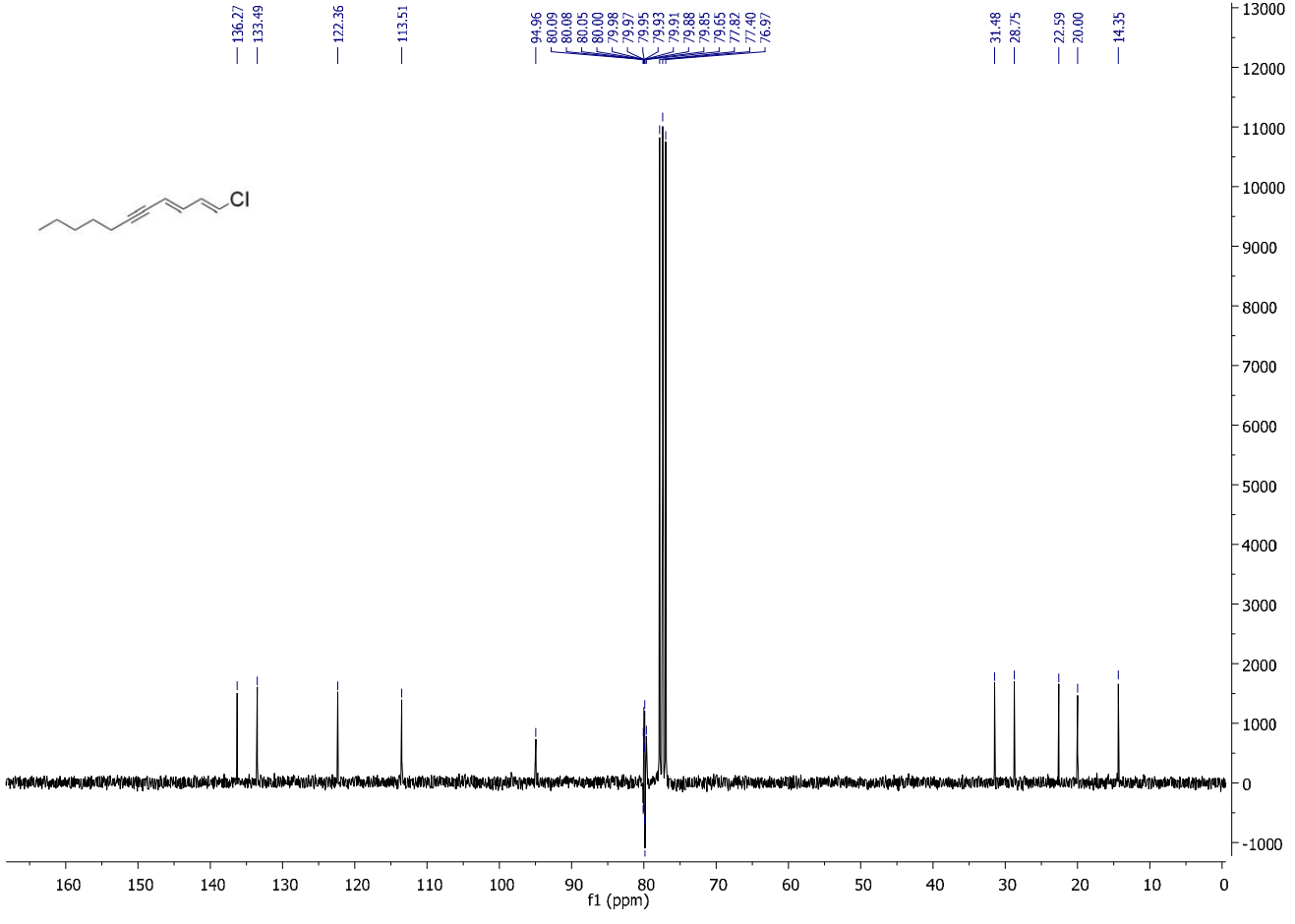
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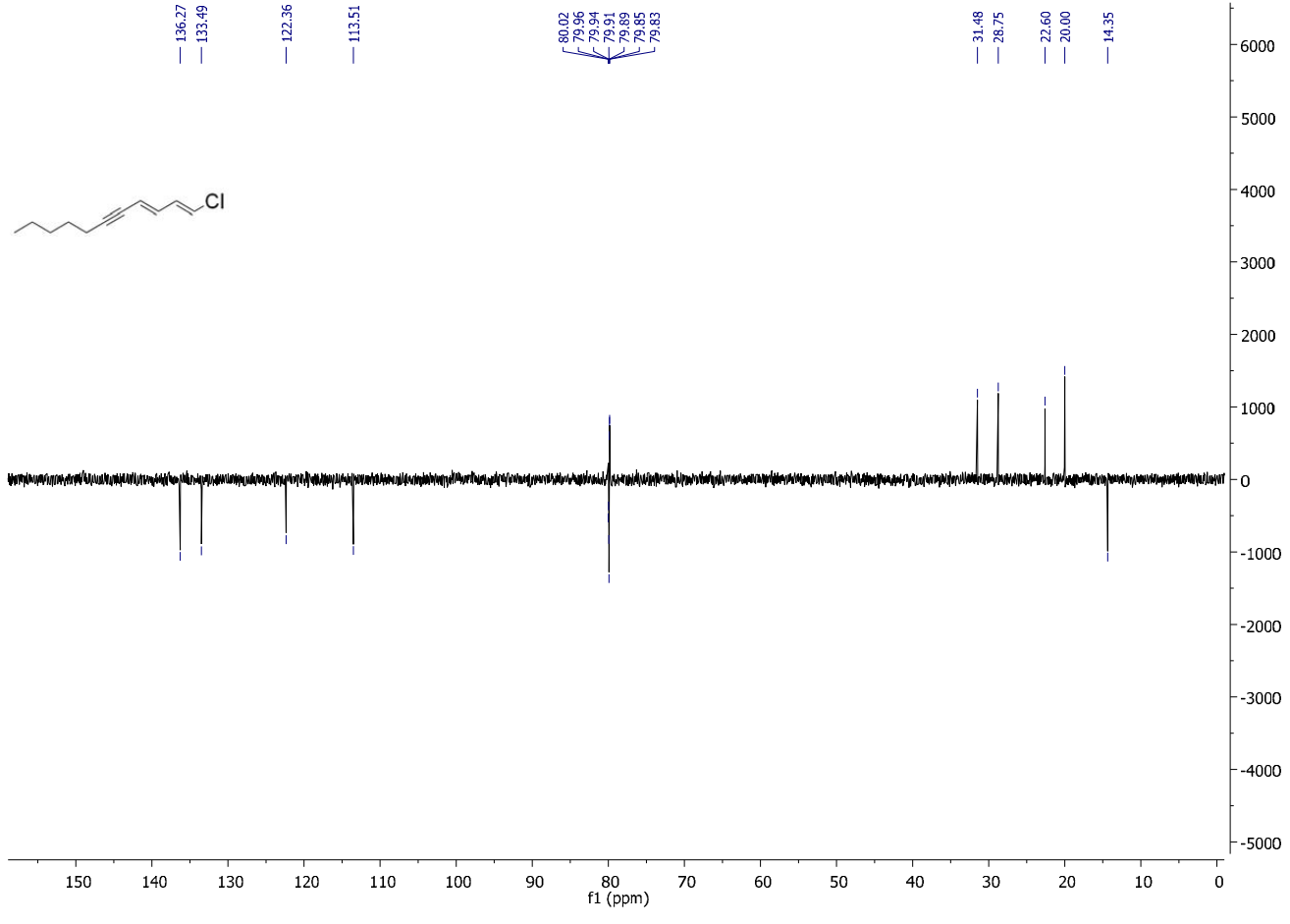
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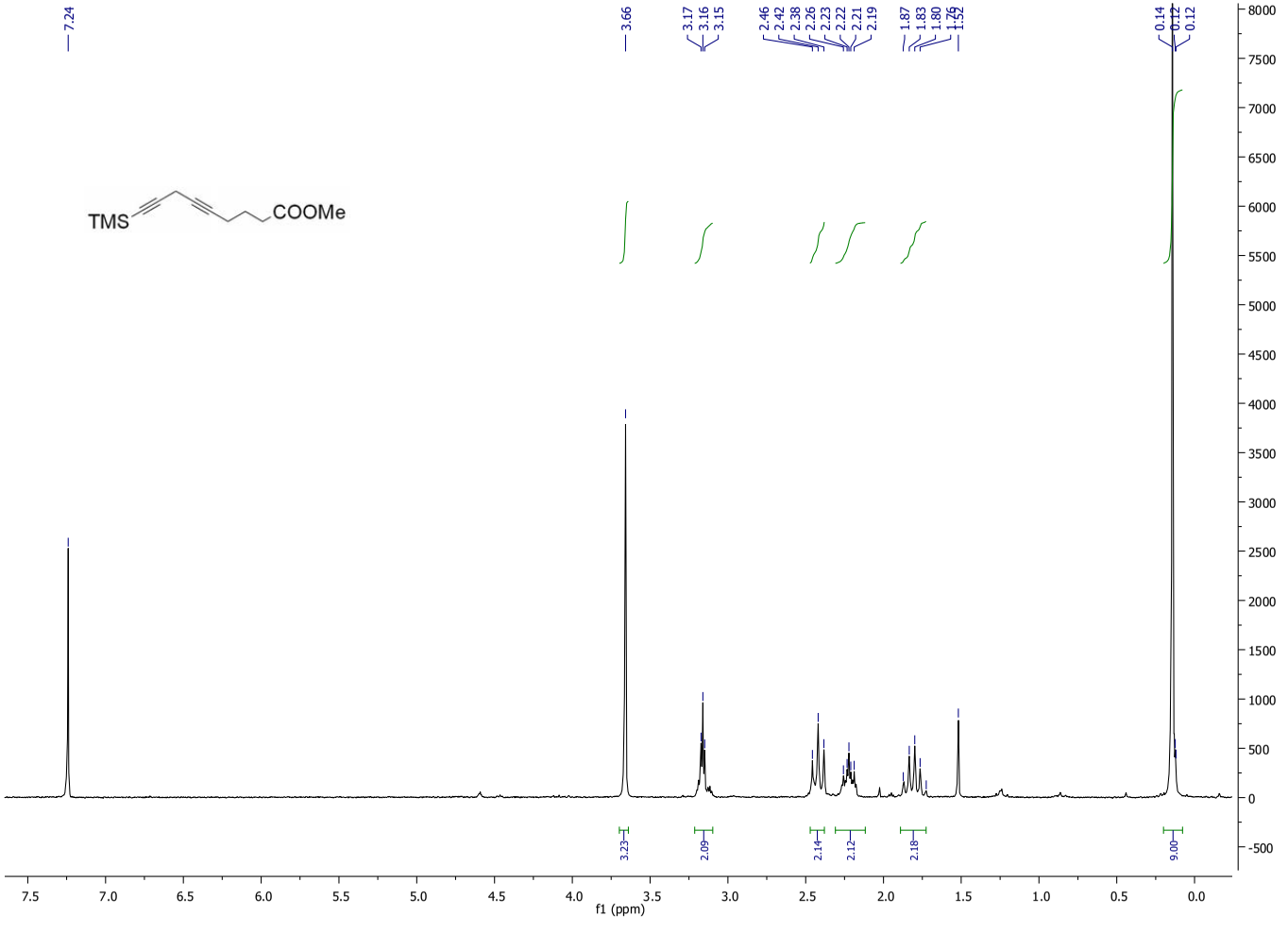
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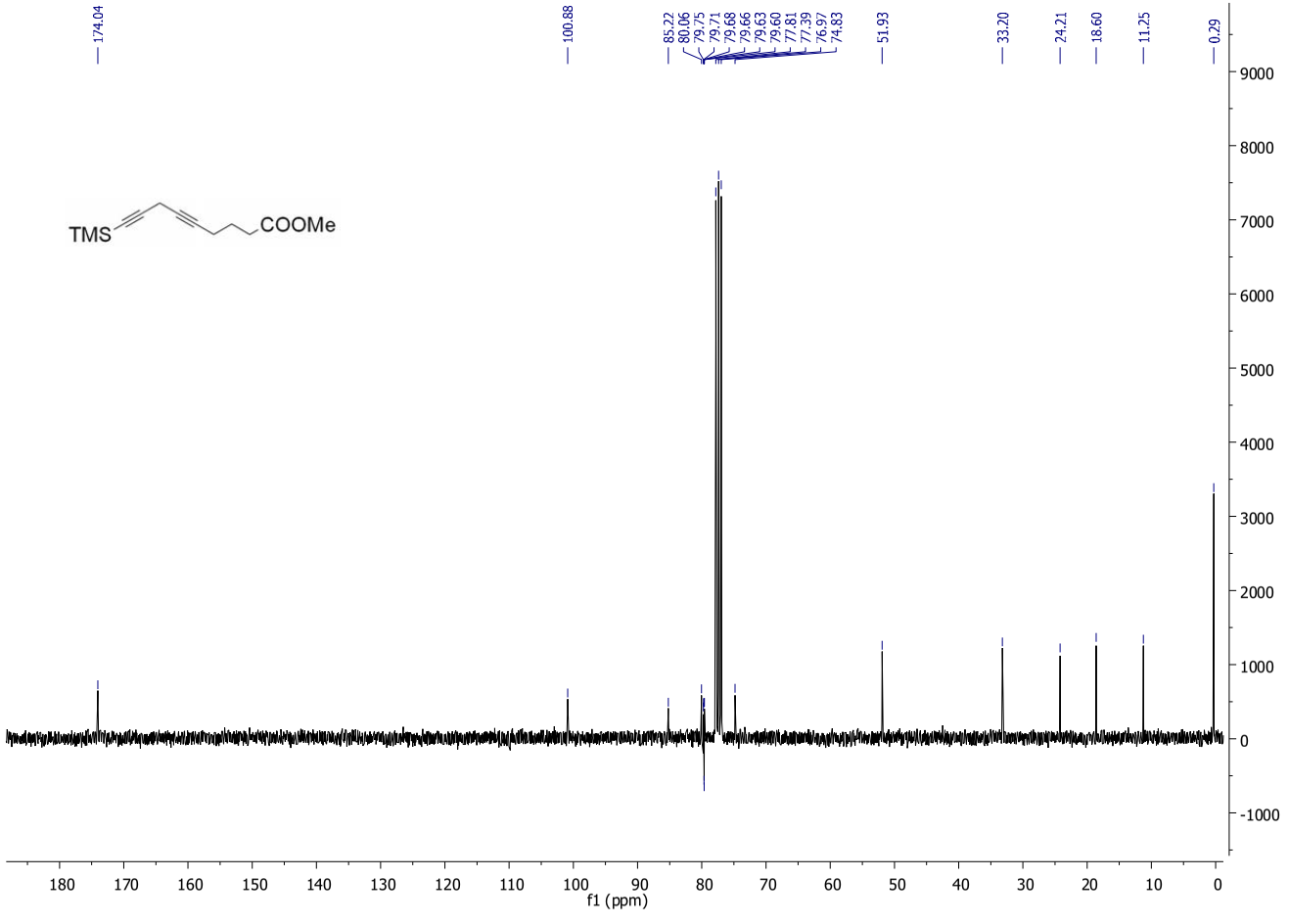
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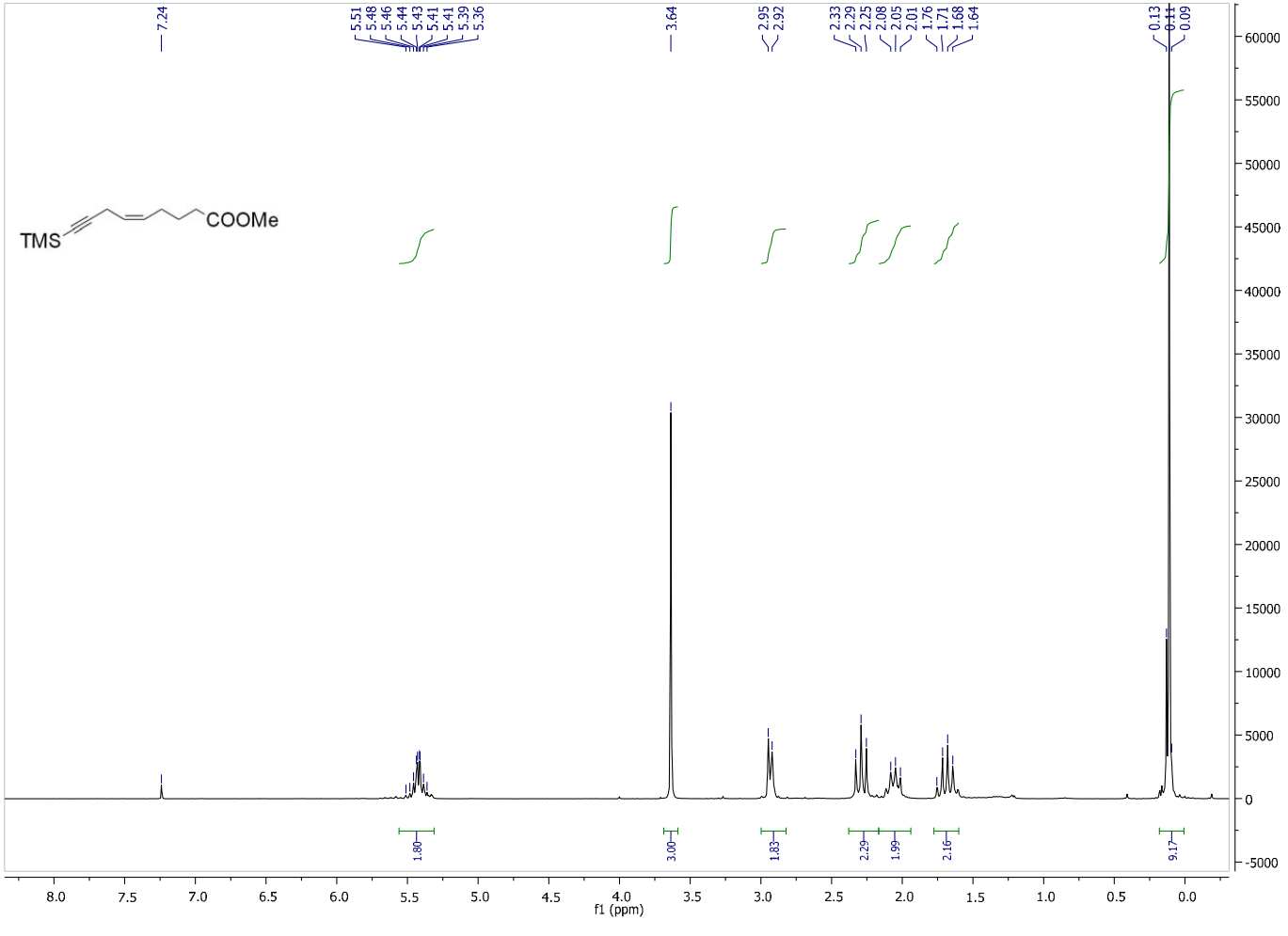
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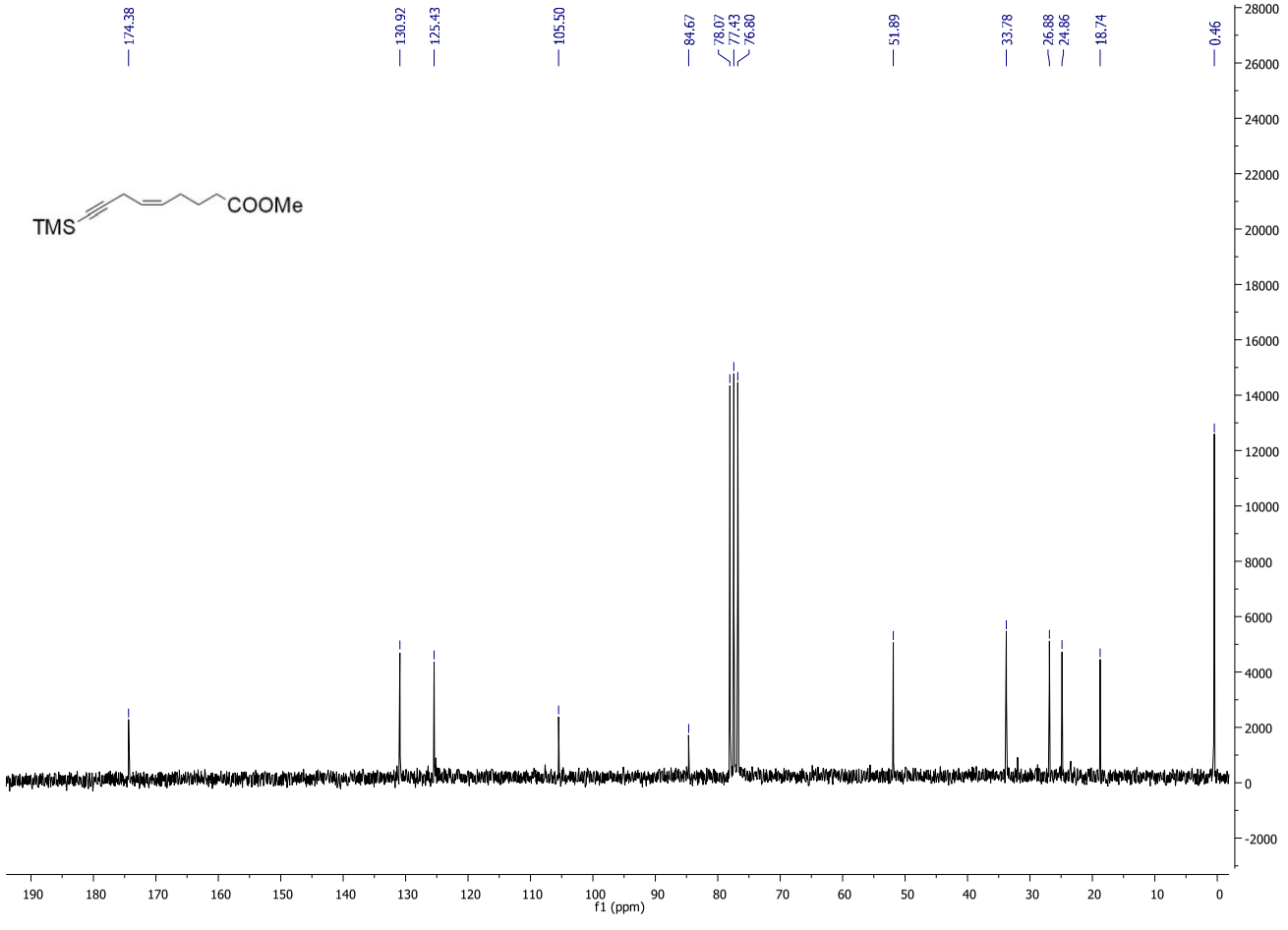
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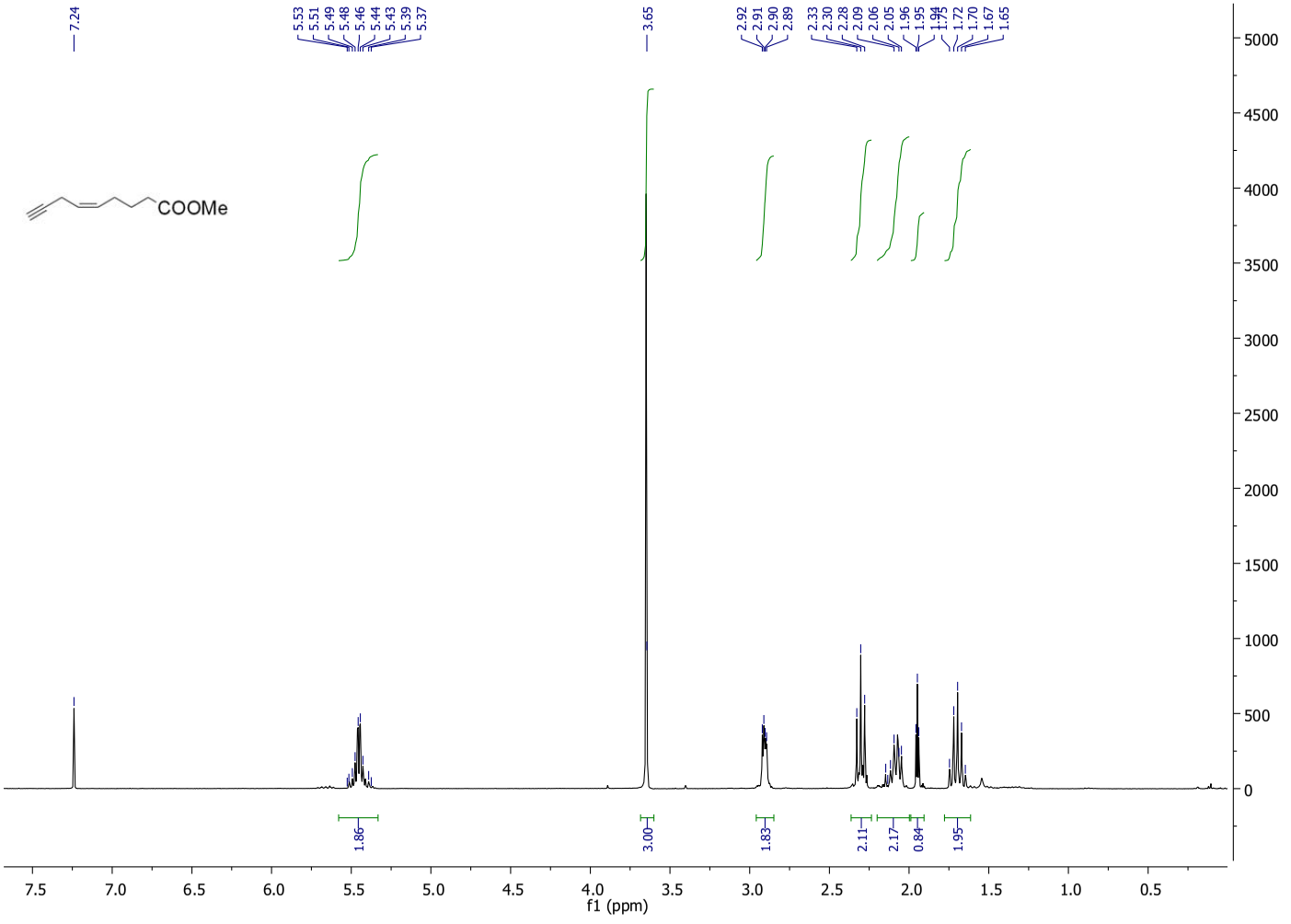
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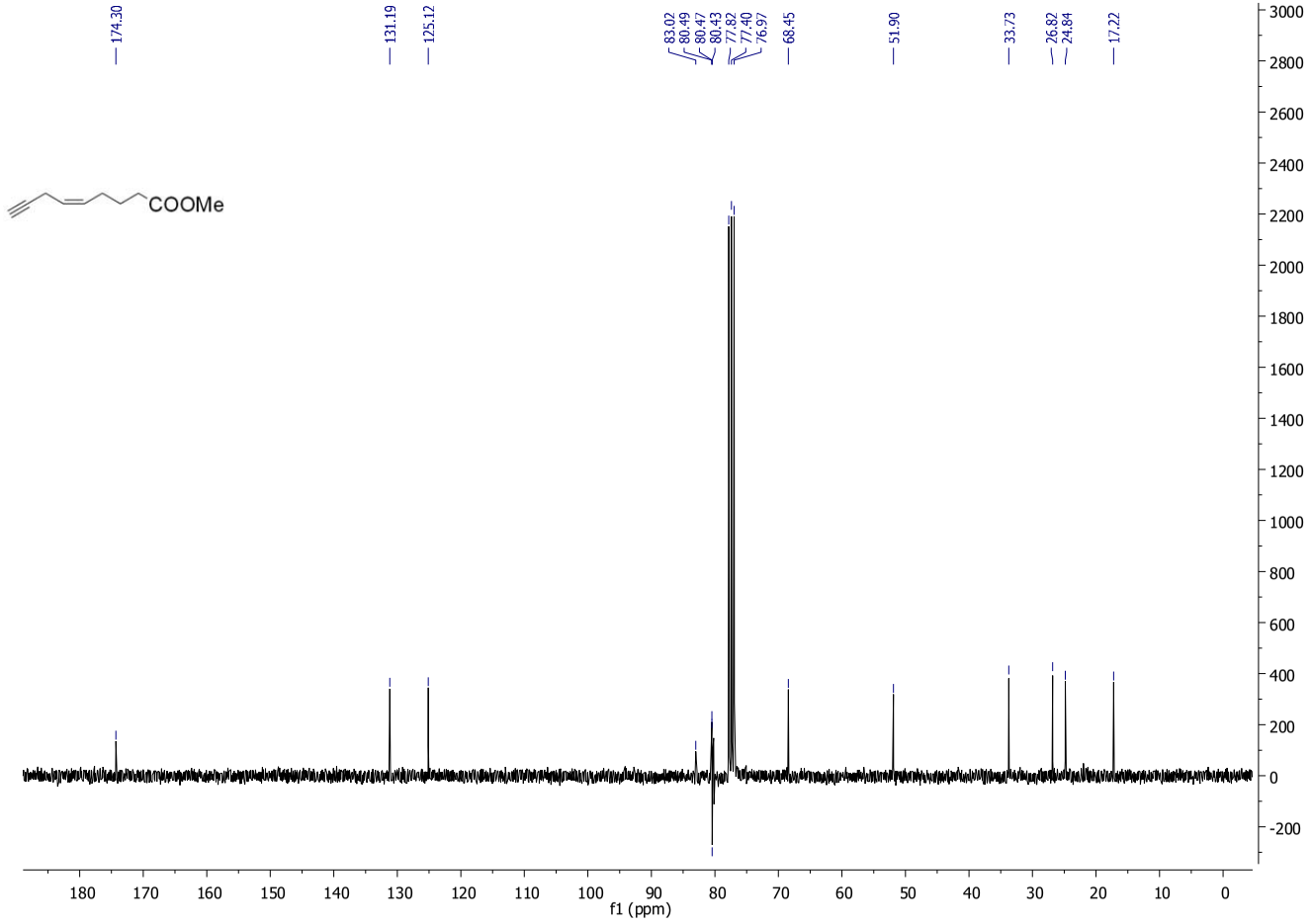
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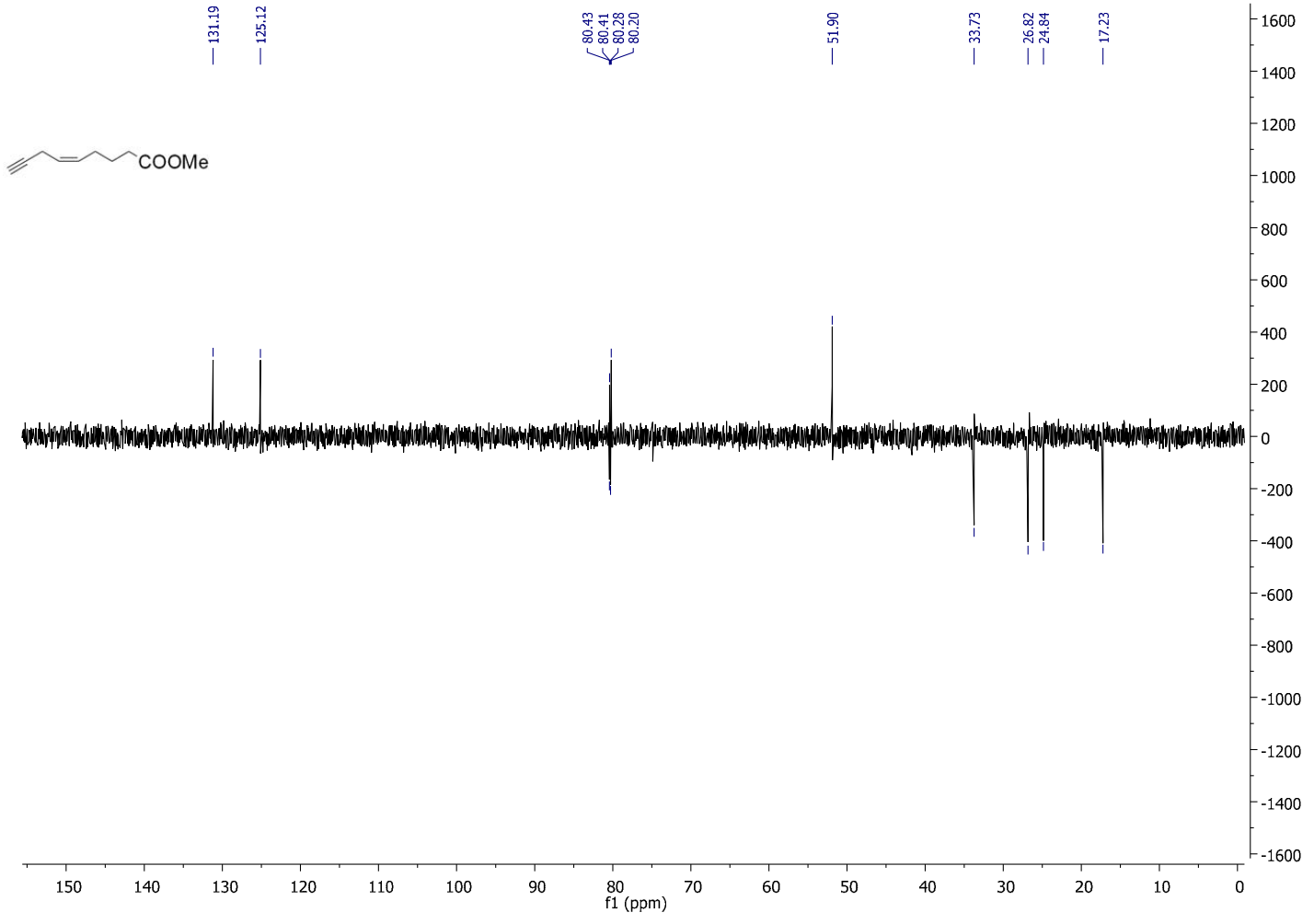
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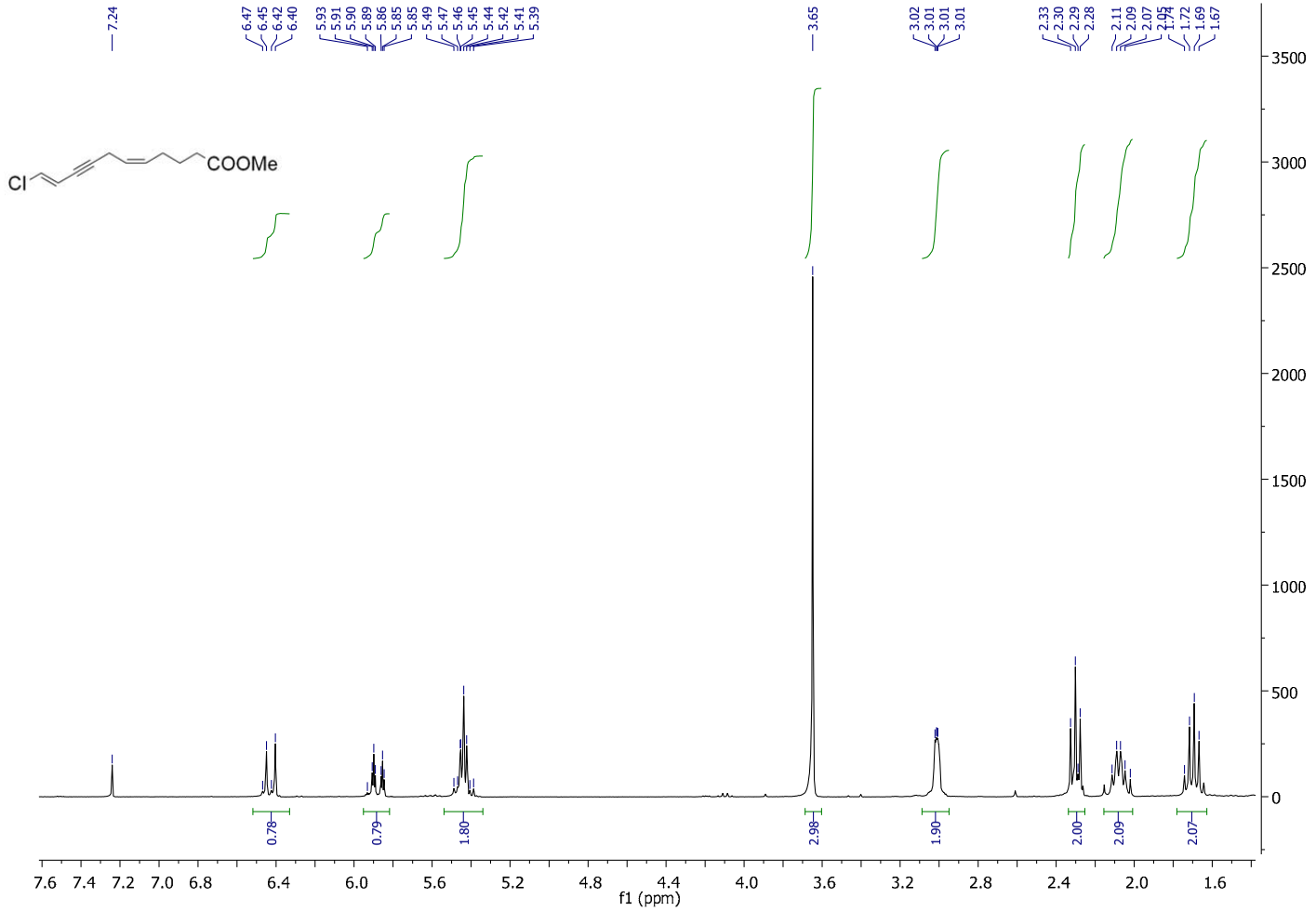
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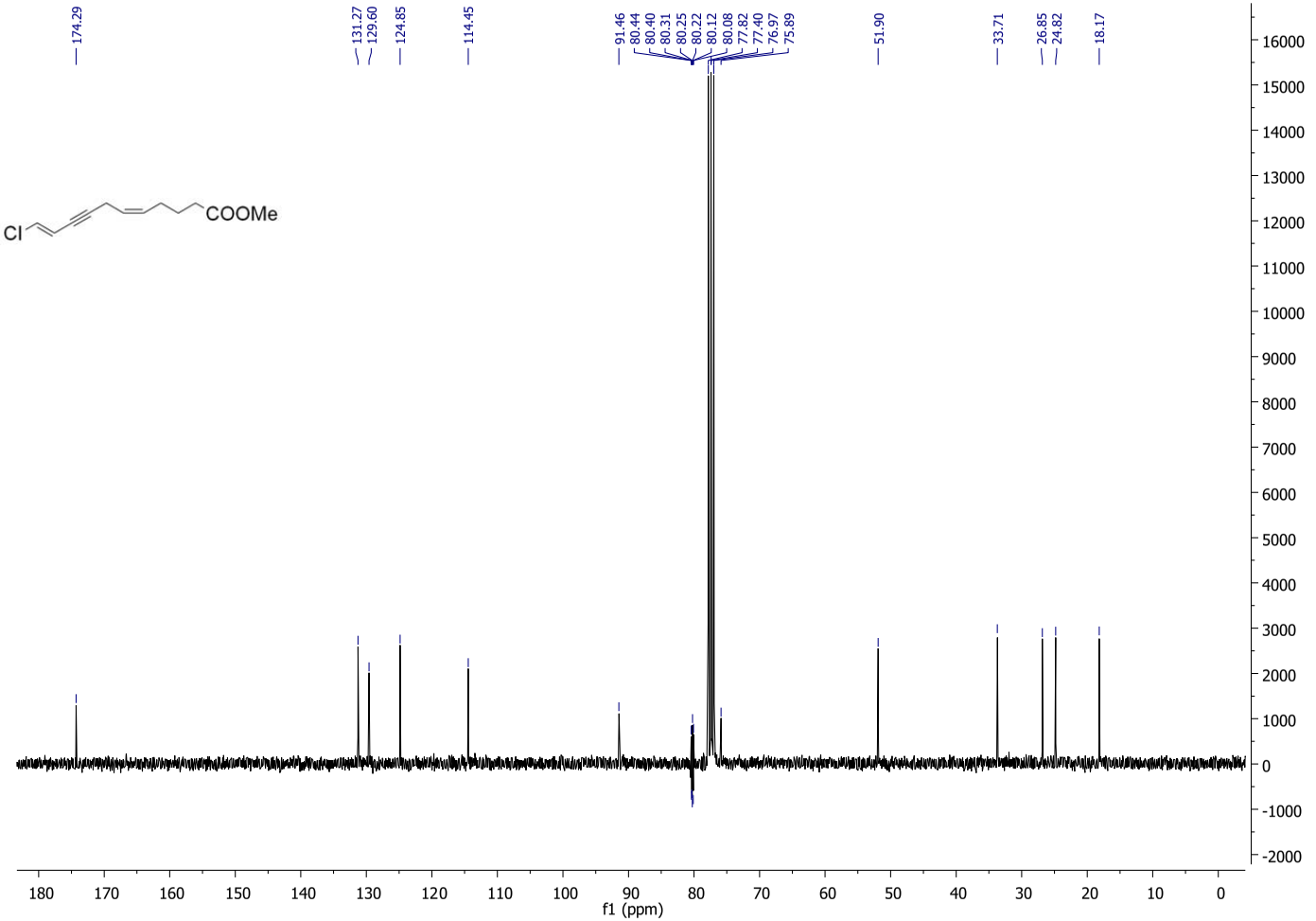
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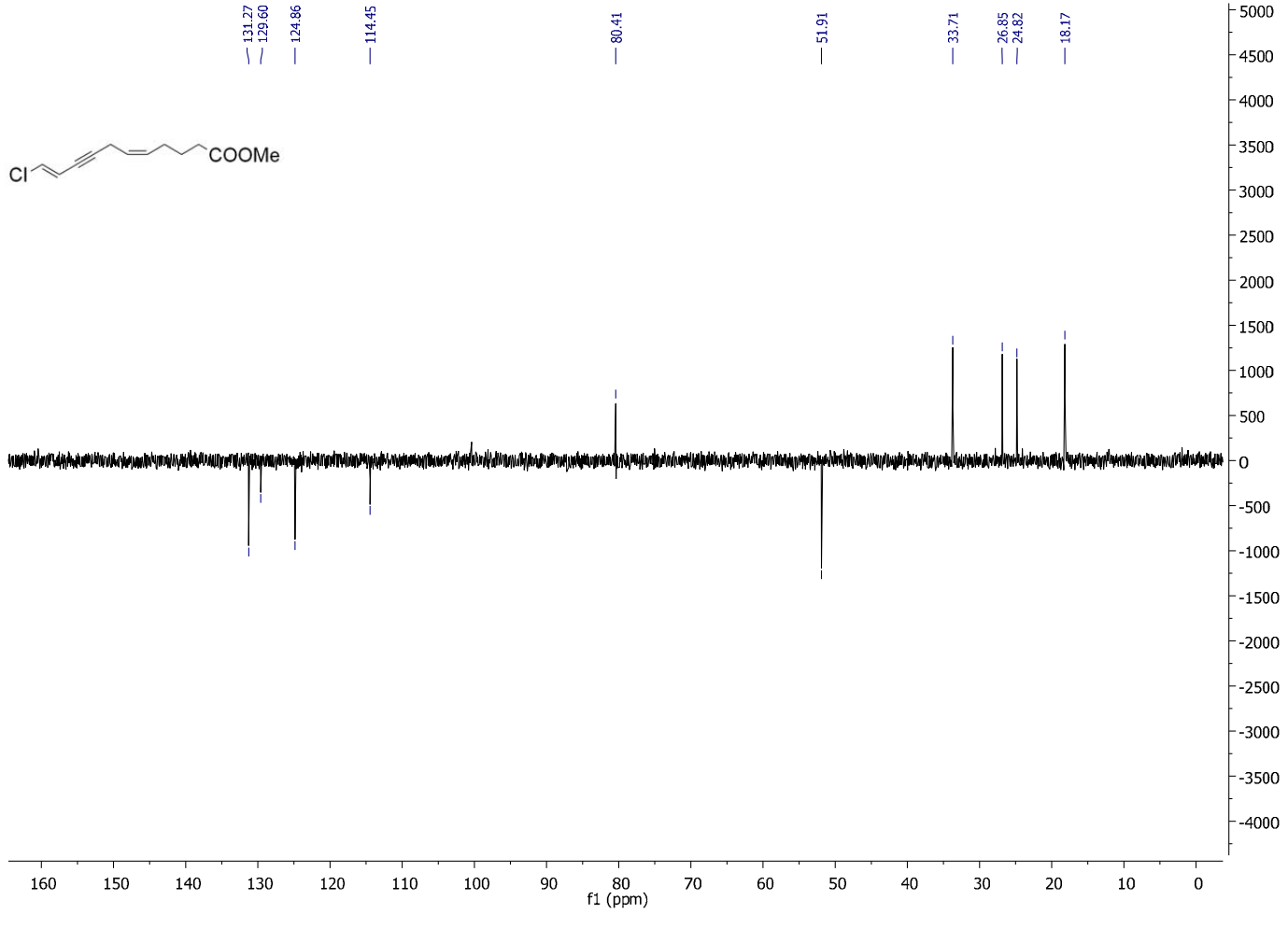
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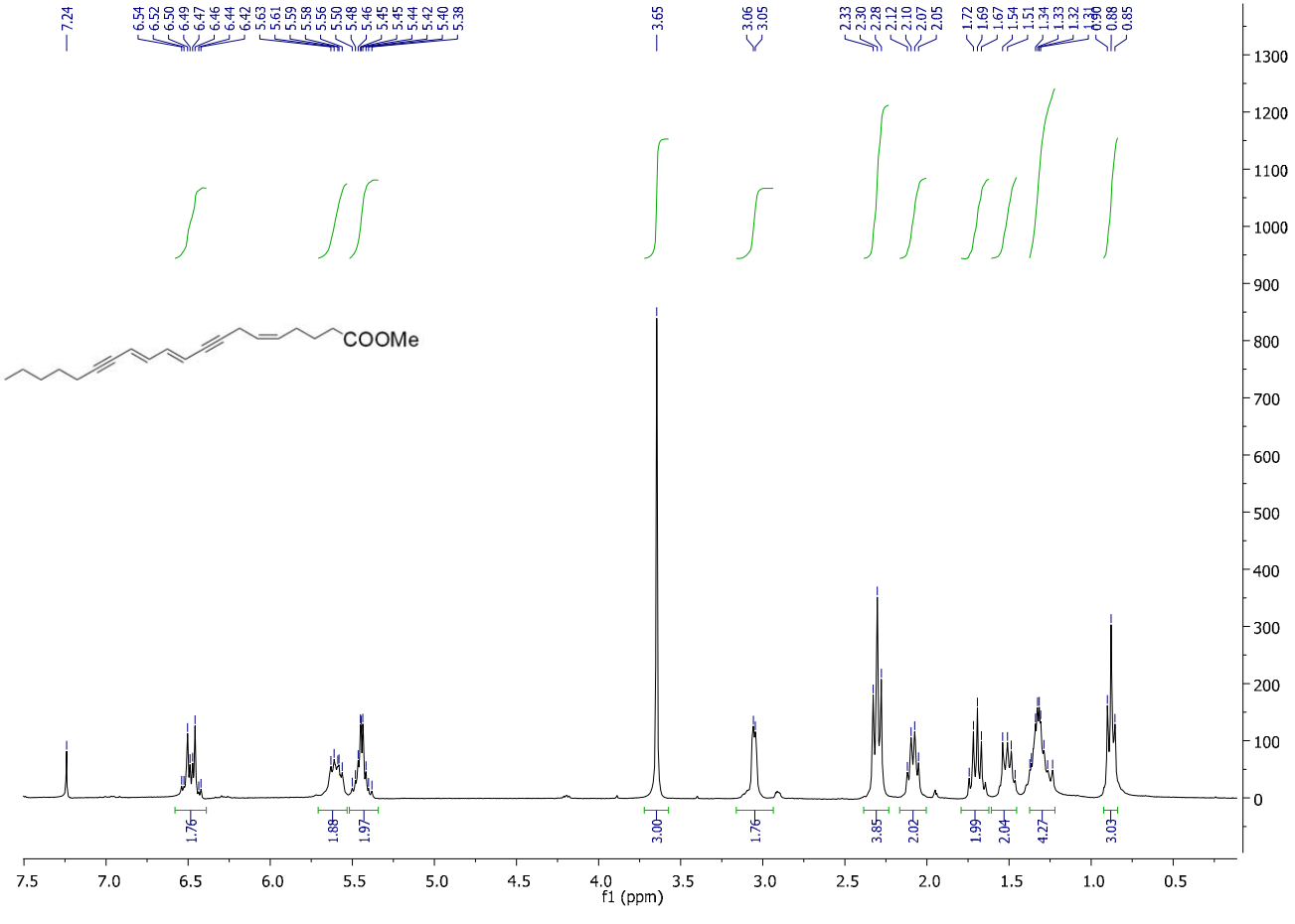
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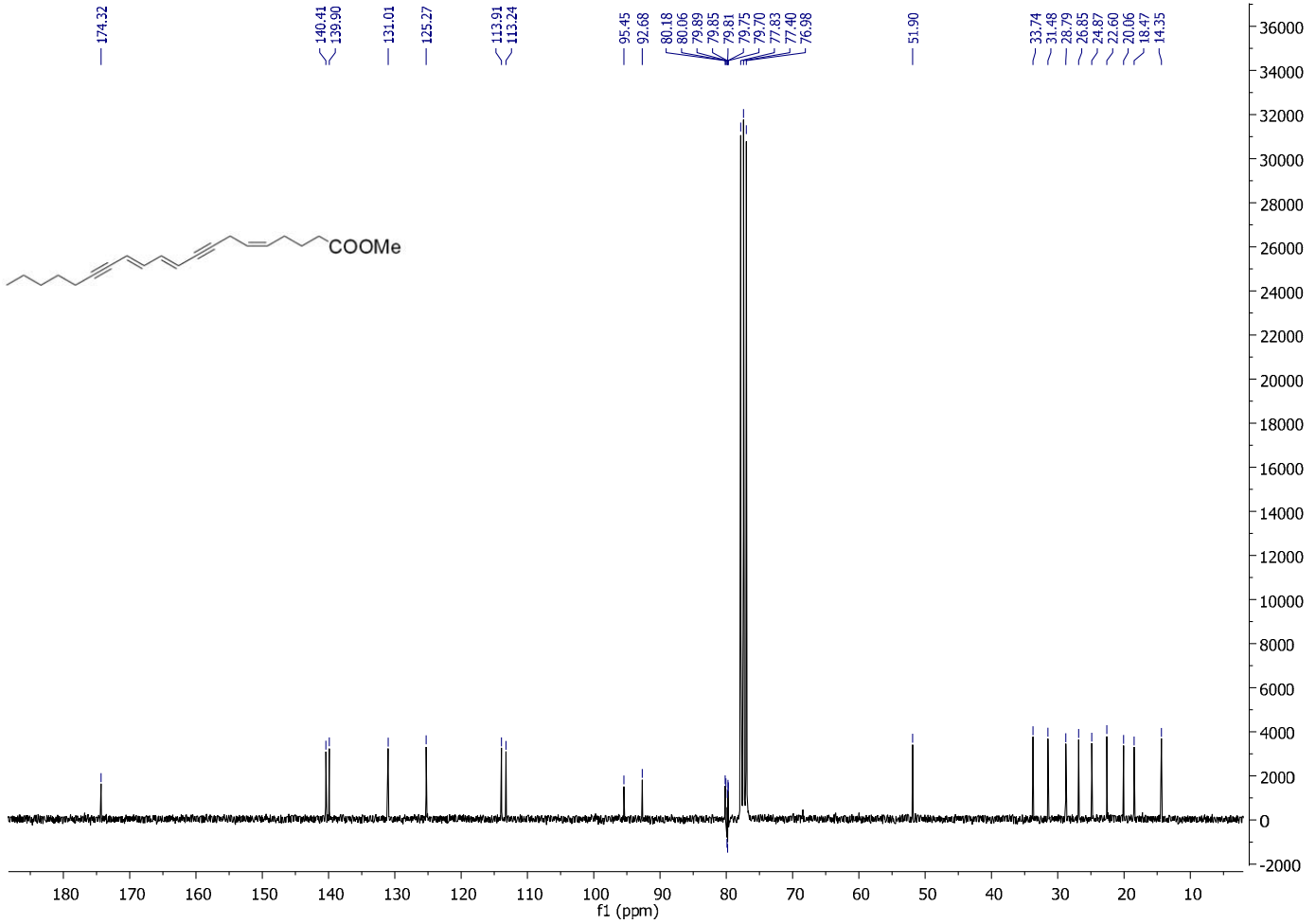
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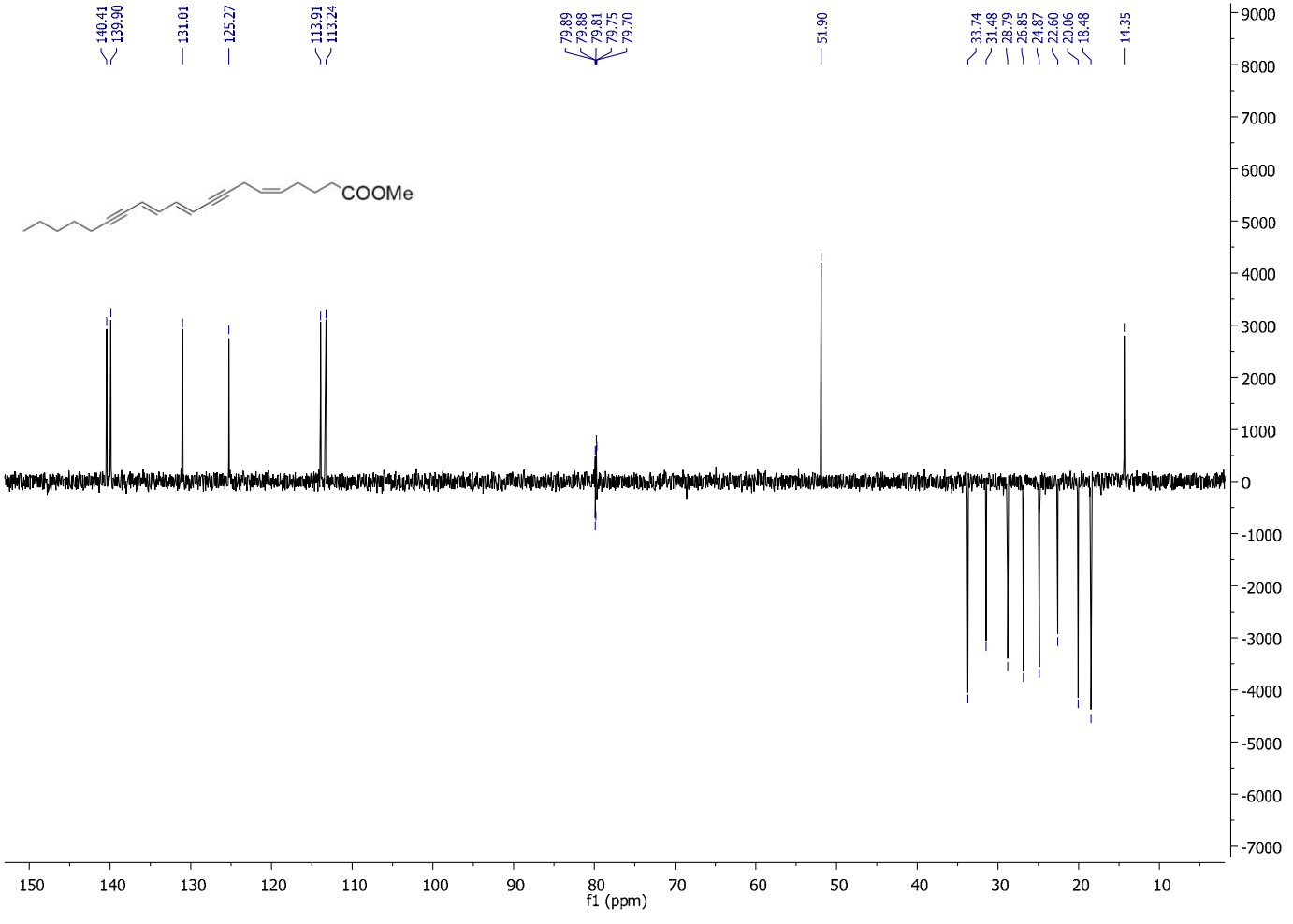
COOMe
f1 (ppm)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79.70
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33.74
31.48
28.79
26.85
24.87
22.60
20.06
18.48
14.35
COOMe
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
-2000
-3000
-4000
-5000
-6000
-7000
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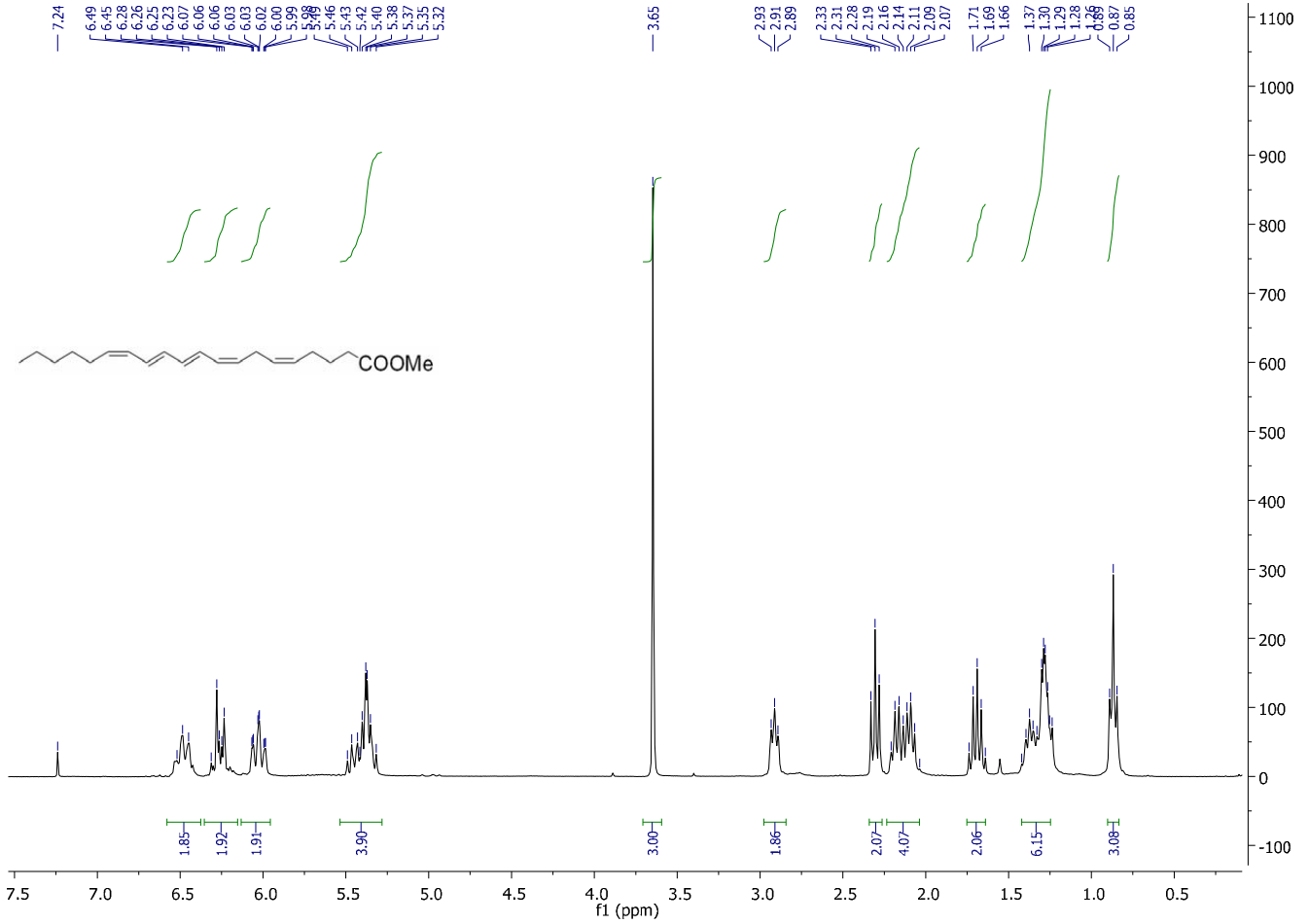
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)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6.03
6.02
6.00
5.99
5.46
5.43
5.42
5.40
5.38
5.37
5.35
5.32
3.65
2.93
2.91
2.89
2.33
2.31
2.28
2.19
2.16
2.14
2.11
2.09
2.07
1.71
1.69
1.66
1.37
1.30
1.29
1.28
0.87
0.85
COOMe
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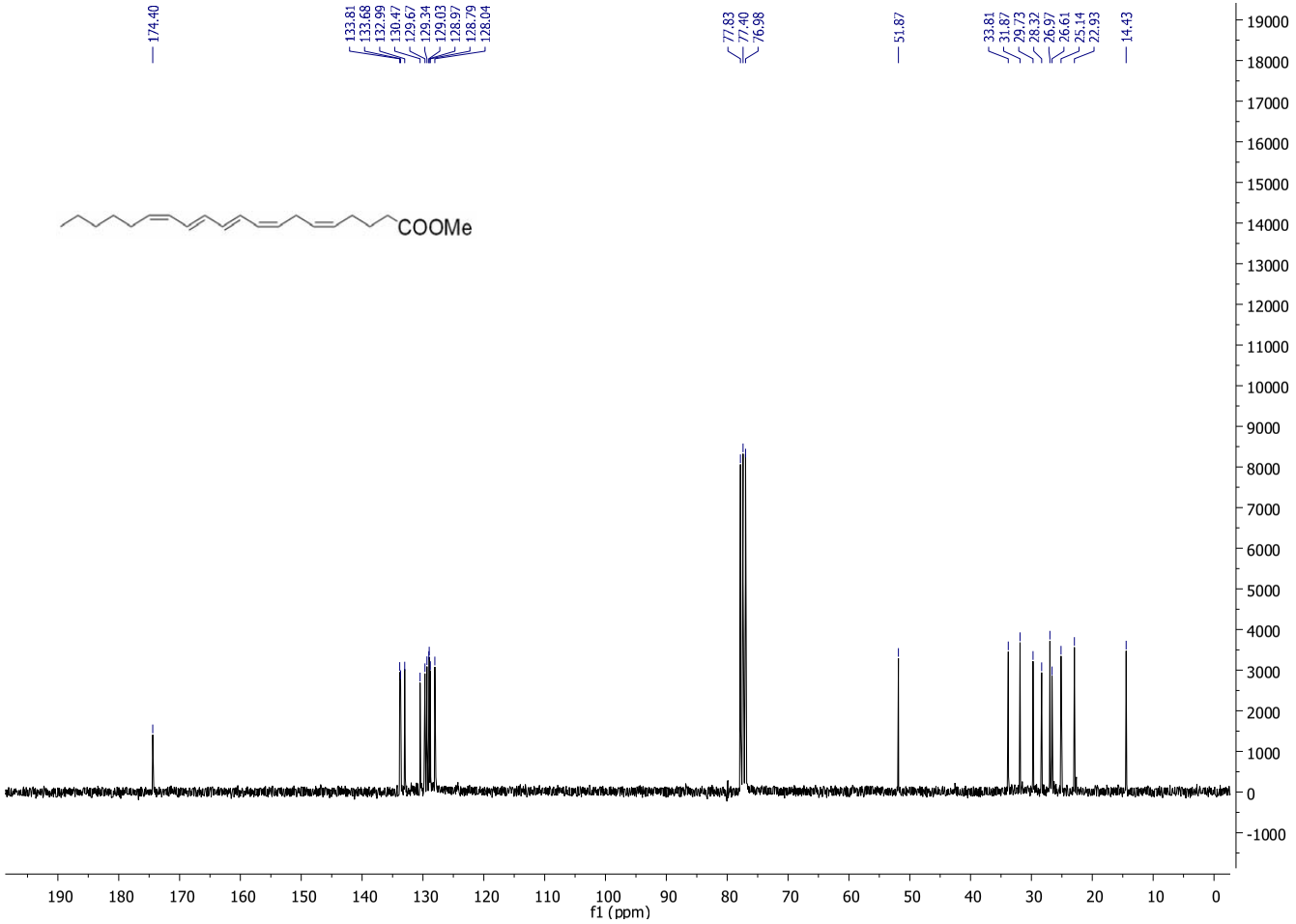
1.86
2.07
4.07
2.06
6.15
3.08
f1 (ppm)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76.98
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26.97
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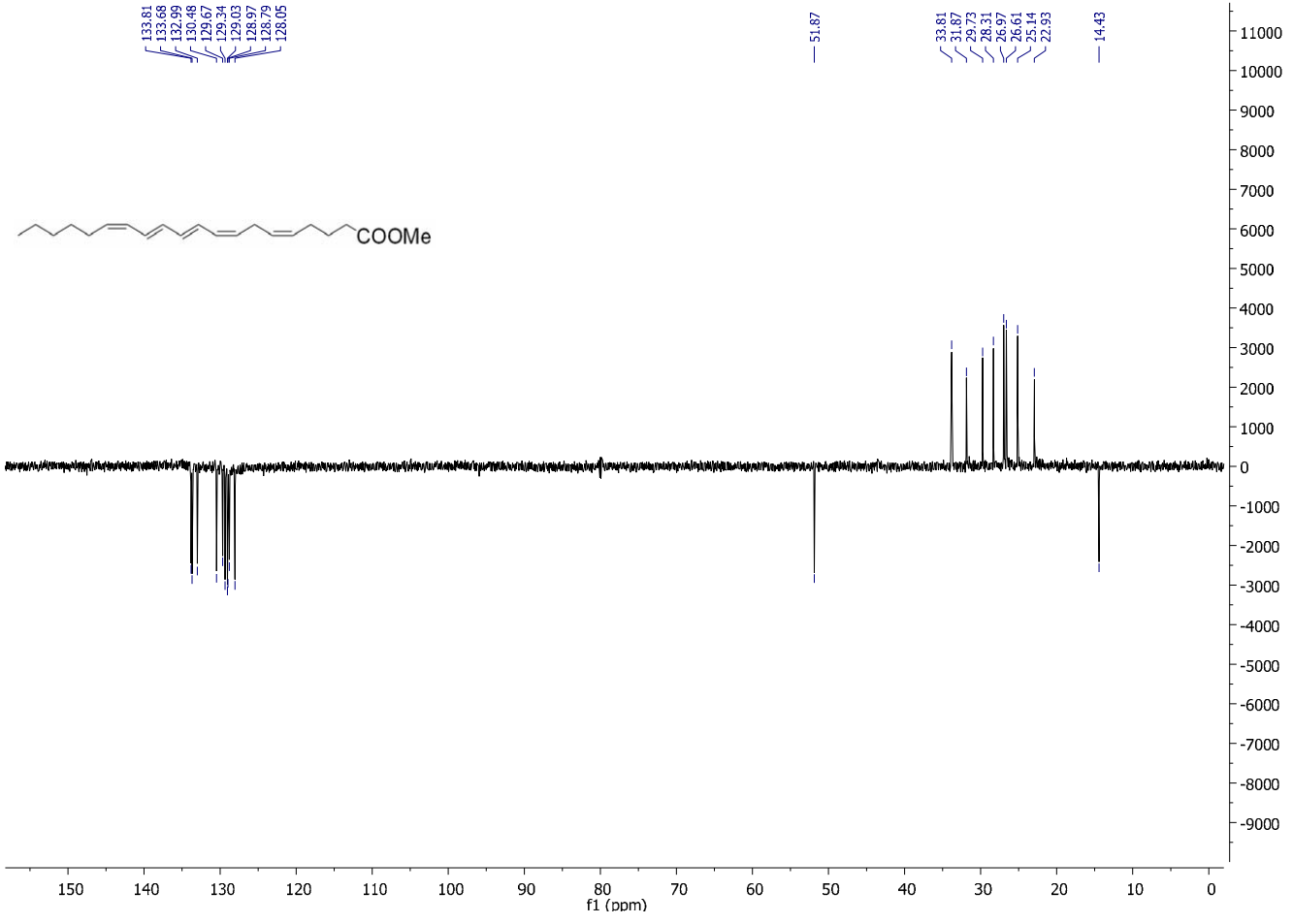
22.93
14.43
COOMe
f1 (ppm)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28.31
26.97
26.61
25.14
22.93
14.43
COOMe
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10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
-2000
-3000
-4000
-5000
-6000
-7000
-8000
-9000
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

Paper IV

Z-Stereoselective semi-reduction of alkynes: Modification of the Boland protocol.

Mohamed, Y. M. A.; Hansen, T. V. *Tetrahedron* **2013**, *69*, under revision.

Supporting Information

Z-Stereoselective semi-reduction of alkynes: Modification of the Boland protocol.

Yasser M. A. Mohamed and Trond Vidar Hansen*

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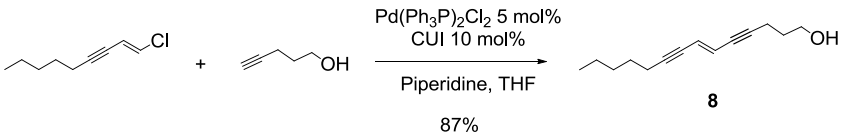
Synthesis of Starting materials

The following compounds wereprepared according to the literature.
(5*Z*,10*E*,12*E*)-Methyl eicosa-5,10,12-trien-8,14-diynoate (**5**),[1] **(*E*)-methyl pentadeca-7-en-5,9-diynoate** (**6**),[2] **(*E*)-hexadeca-8-en-6,10-diyne** (**10**),[3] **(*E*)-oct-6-en-4-yn-1-ol** (**14**),[4] **(*E*)-hex-4-en-2-yn-1-ol**(**16**),[5] **(*E*)-pent-3-en-1-yn-1-ylbenzene** (**18**),[4] **1-(4-(phenylethynyl)-phenyl)-ethanone** (**24**),[6] **1-nitro-4-(phenylethynyl)benzene** (**26**),[7] **2-(hept-1-yn-1-yl)thiophene** (**29**).[8]

(*E*)-Tetradeca-6-en-4,8-diyn-1-ol (8):

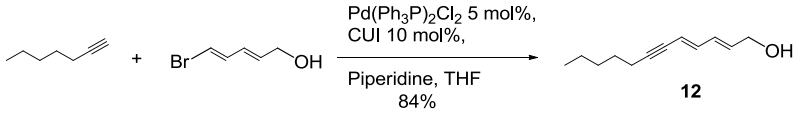
To a mixture of $Pd(Ph_3P)_2Cl_2$ (45 mg, 0.064 mmol, 5 mol%) and CuI (24 mg, 0.128 mmol, 10 mol%) in THF (5 ml) under argon, piperidine (0.38 ml, 3.84 mmol, 3 eq) and (*E*)-1-chlorononon-1-en-3-yne (200 mg, 1.28 mmol, 1 eq) were added, followed by the addition of 4-pentyn-1-ol (323 mg, 3.84 mmol, 3 eq). The reaction mixture was allowed to stir for 3 h at room temperature. The resulting mixture was diluted with EtOAc (10 ml) then filtered through short pad of silica gel using EtOAc (30 ml) as eluent. The solution was washed with saturated ammonium chloride, dried over $MgSO_4$ and evaporated under reduced pressure. The resulting crude product was purified by column chromatography (silica gel, hexane/EtOAc, 90:10) to afford the title product as a colorless oil (227 mg, 87%).



^{1}H NMR (300 MHz, $CDCl_3$): δ= 5.86 (s, 2H), 3.65(t, J = 6.5 Hz, 2H), 2.44-2.35 (m, 4H), 2.29 (td, J = 7.1, 1.6 Hz, 2H), 1.84 (q, J = 7.1 Hz, 2H), 1.53-1.48 (m, 4H), 1.36-1.28 (m, 2H), 0.88 (t, J = 7.1 Hz, 3H).^{13}C NMR (75 MHz, $CDCl_3$): δ=120.25 x 2 CH, 95.74, 93.62, 79.41, 74.89, 62.74, 33.22, 31.47, 28.68, 22.58, 19.98, 19.46, 14.35.

(2*E*,4*E*)-Dodeca-2,4-dien-6-yn-1-ol (12):

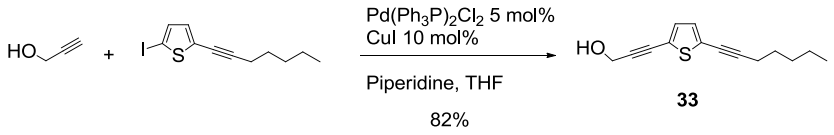
To a mixture of $Pd(Ph_3P)_2Cl_2$ (25 mg, 0.036 mmol, 5 mol%) and CuI (13 mg, 0.069 mmol, 10 mol%) in THF (3 ml) under argon, piperidine (0.204 ml, 2.07 mmol, 3 eq) and (2*E*,4*E*)-5-bromopenta-2,4-dien-1-ol (113 mg, 0.69 mmol, 1 eq) were added, followed by the addition of 1-heptyne (73 mg, 0.76 mmol, 1.1 eq). The reaction mixture was allowed to stir for 3 h at room temperature. The resulting mixture was diluted with EtOAc (5 ml) then filtered through short pad of silica gel using EtOAc (10 ml) as eluent. The solution was washed with saturated ammonium chloride, dried ($MgSO_4$) and evaporated under reduced pressure. The resulting crude product was purified by column chromatography (silica gel, hexane/EtOAc, 90:10) to afford the title product as a colorless oil (104 mg, 84%).



^{1}H NMR (300 MHz, $CDCl_3$): δ= 6.53-6.44 (m, 1H), 6.30-6.20 (m, 1H), 5.99-5.80 (m, 1H), 5.62 - 5.40 (m, 1H), 4.20 (d, J = 5.5 Hz, 2H), 2.31 (qd, J = 7.2, 1.5 Hz, 2H), 1.54-1.30 (m, 6H), 0.88 (t, J = 6.8 Hz, 3H).^{13}C NMR (75 MHz, $CDCl_3$): δ= 139.91, 134.82, 130.85, 112.77, 94.06, 79.95, 63.51, 31.48, 28.83, 22.60, 19.99, 14.36.

3-(5-(Hept-1-yn-1-yl)thiophen-2-yl)prop-2-yn-1-ol (31):

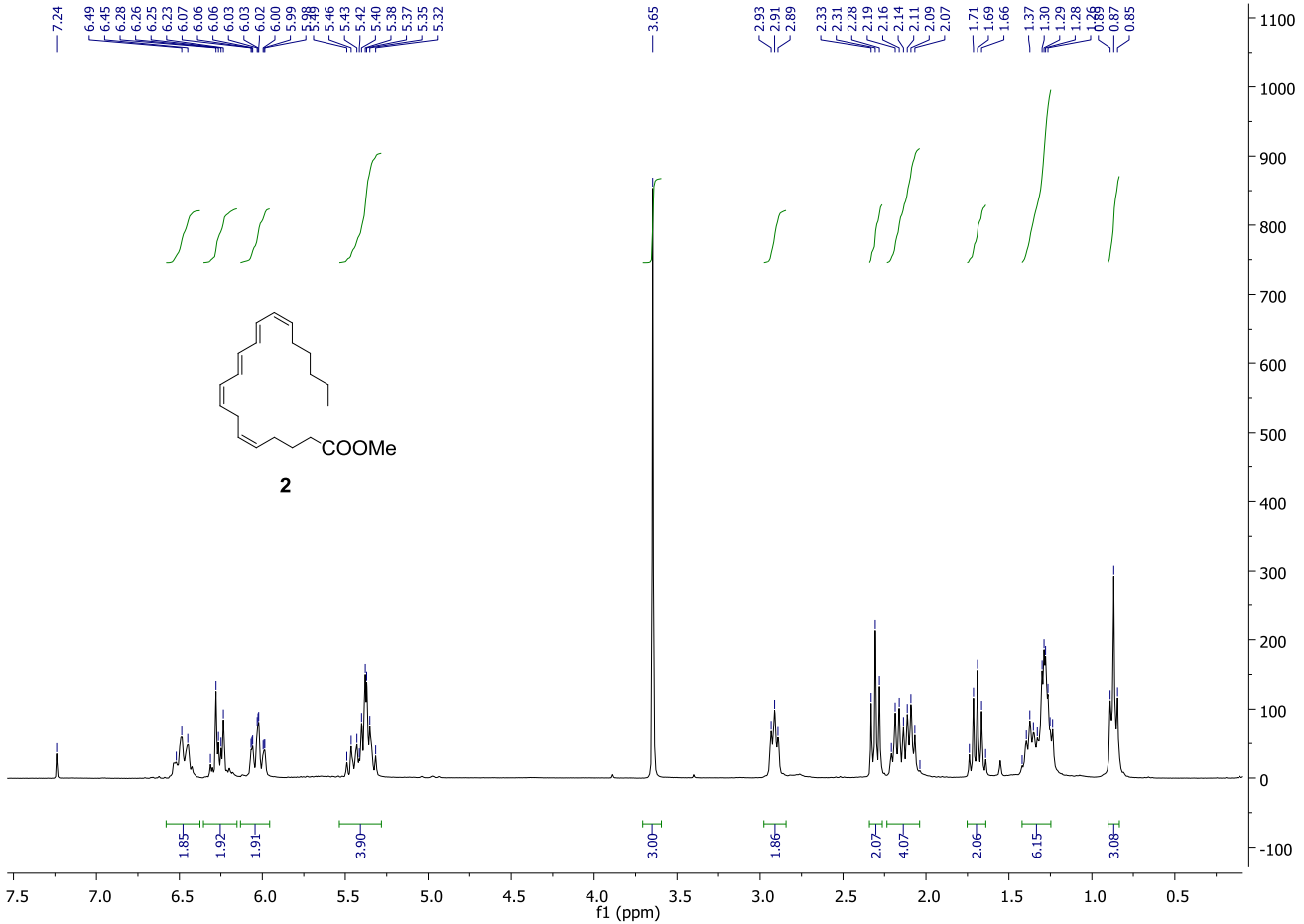
To a mixture of $Pd(Ph_3P)_2Cl_2$ (103 mg, 0.148 mmol, 5 mol%) and CuI (57 mg, 0.296 mmol, 10 mol%) in THF (5 ml) under argon, piperidine (0.875 ml, 8.88 mmol, 3 eq) and 2-(hept-1-yn-1-yl)-5-iodothiophene (900 mg, 2.96 mmol, 1 eq) were added, followed by the addition of propargyl alcohol (497 mg, 8.88 mmol, 3 eq). The reaction mixture was allowed to stir for 3 h at room temperature. The resulting mixture was diluted with EtOAc (10 ml) then filtered through short pad of silica gel using EtOAc (30 ml) as eluent. The solution was washed with saturated ammonium chloride, dried ($MgSO_4$) and evaporated under reduced pressure. The resulting crude product was purified by column chromatography (silica gel, hexane/EtOAc, 90:10) to afford the title product as a colorless oil (563 mg, 82%).



^{1}H NMR (300 MHz, $CDCl_3$): δ= 7.00 (d, J = 3.8 Hz, 1H), 6.92 (d, J = 3.8 Hz, 1H), 4.47 (s, 3H), 2.39 (t, J = 7.1 Hz, 1H), 1.59 (td, J = 14.4, 12.9, 5.7 Hz, 1H), 1.44-1.23 (m, 2H), 0.90 (t, J = 7.1 Hz, 1H).^{13}C NMR (75 MHz, $CDCl_3$): δ= 132.50, 131.06, 126.43, 122.59, 96.30, 91.64, 79.23, 73.63, 52.12, 31.51, 28.54, 22.59, 20.08, 14.35.

References

1. Mohamed, Y. M. A.; Hansen, T. V. *Tetrahedron Lett*.,**2011**, *52*, 1057-1059.
2. Alami, M.; Crousse, B.;Linstrumelle, G. *Tetrahedron Lett*. **1994**, *35*, 3543-3544.
3. Carpita, A.; Rossi, R. *Tetrahedron Lett*. **1986**, *27*, 4351-4354.
4. Feuerstein, M. ; Chahen, L.; Doucet, H. ; Santelli, M. *Tetrahedron* **2005**, *62*, 112-120.
5. Crook, K. E.;Nie, J.; Welker, M. E. *Lett. Org. Chem.* **2008**,*5*, 158-164.
6. Thorand, S.; Krause, N. *J. Org. Chem*. **1998**, *63*, 8551-8553.
7. Fairlamb, I. J. S.; Lee, A. F.; Loe-Mie, F. E. M.; Niemelä, E. H.; O'Brien, C. T.; Whitwood A. C. *Tetrahedron* **2005**, *61,* 9827-9838.
8. de Sousa Jr, P. T.; Taylor, R. J. K. *J. Braz. Chem. Soc*. **1993**, *4*, 64-86.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2.91
2.89
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2.31
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2.09
2.07
1.71
1.69
1.66
1.37
1.30
1.29
1.28
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0.85
COOMe
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4.07
2.06
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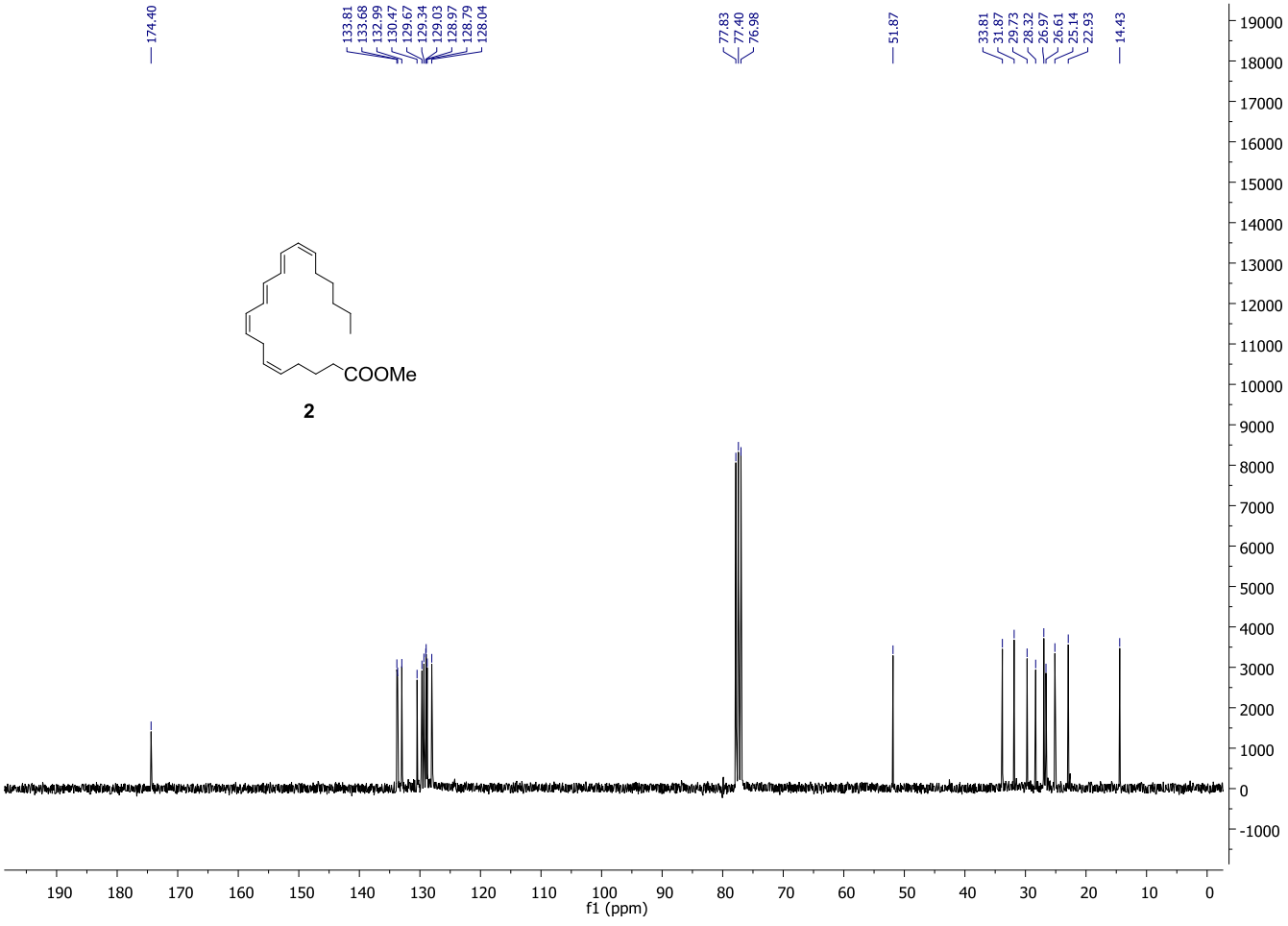
3.08
f1 (ppm)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76.98
51.87
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22.93
14.43
COOMe
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f1 (ppm)

Data File D:\DATA\YASSER\0000017.D

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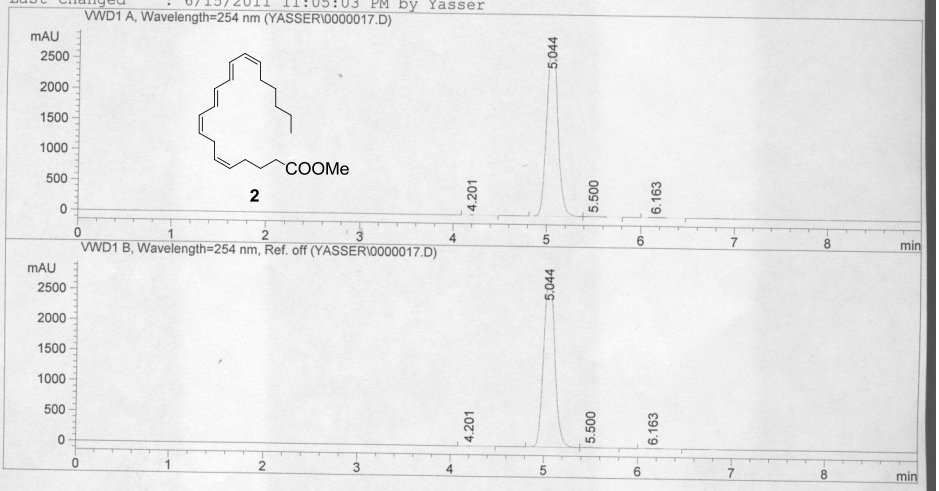

Area Percent Report

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Dilution : 1.0000
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Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=254 nm

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2	5.044	BV	0.1310	2.34195e4	2800.78540	99.4448
3	5.500	VB	0.1355	80.94923	8.76152	0.3437
4	6.163	BB	0.1410	34.40047	3.68333	0.1461
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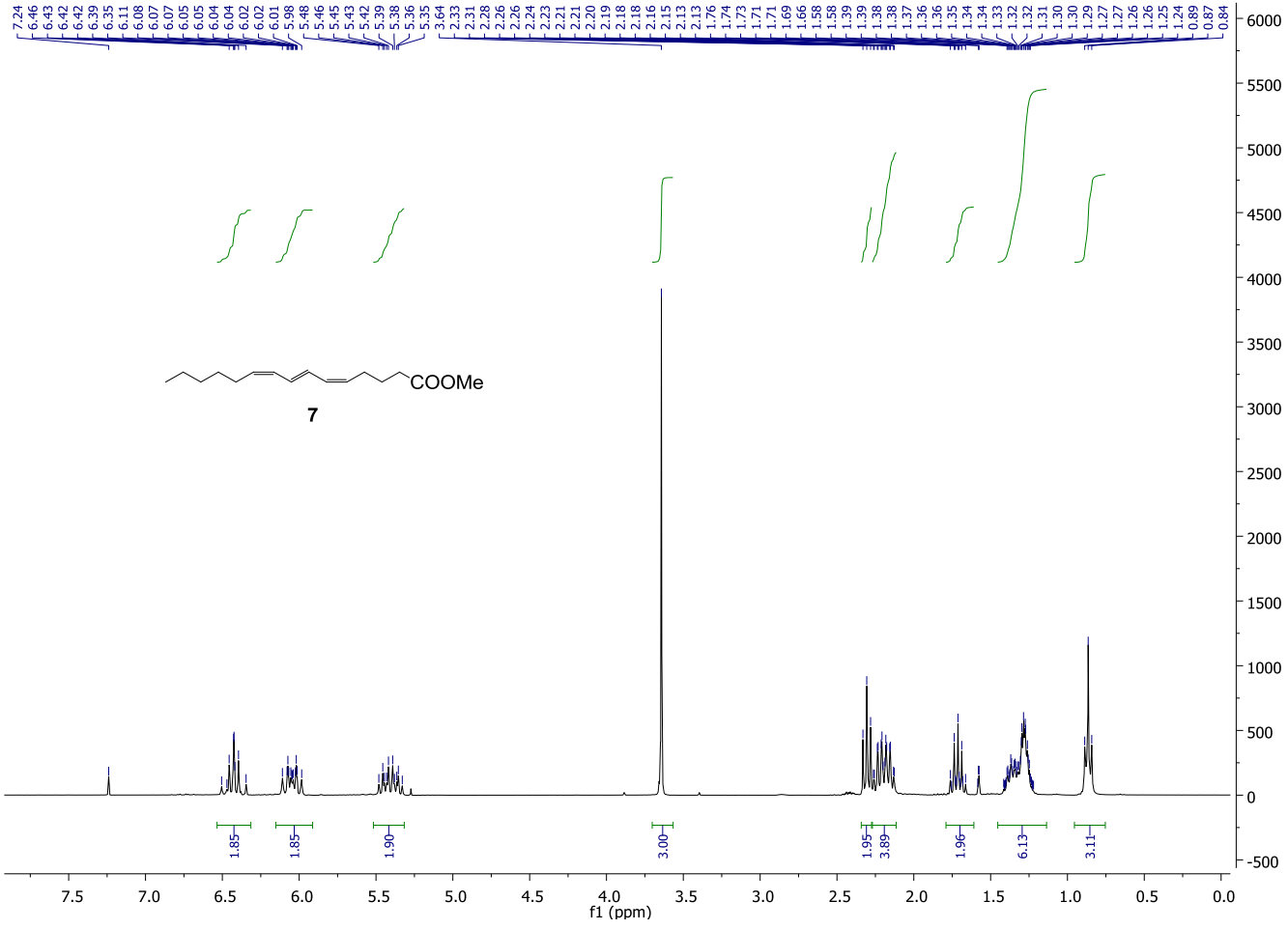
nstrument 1 6/15/2011 11:05:15 PM Yasser


COOMe
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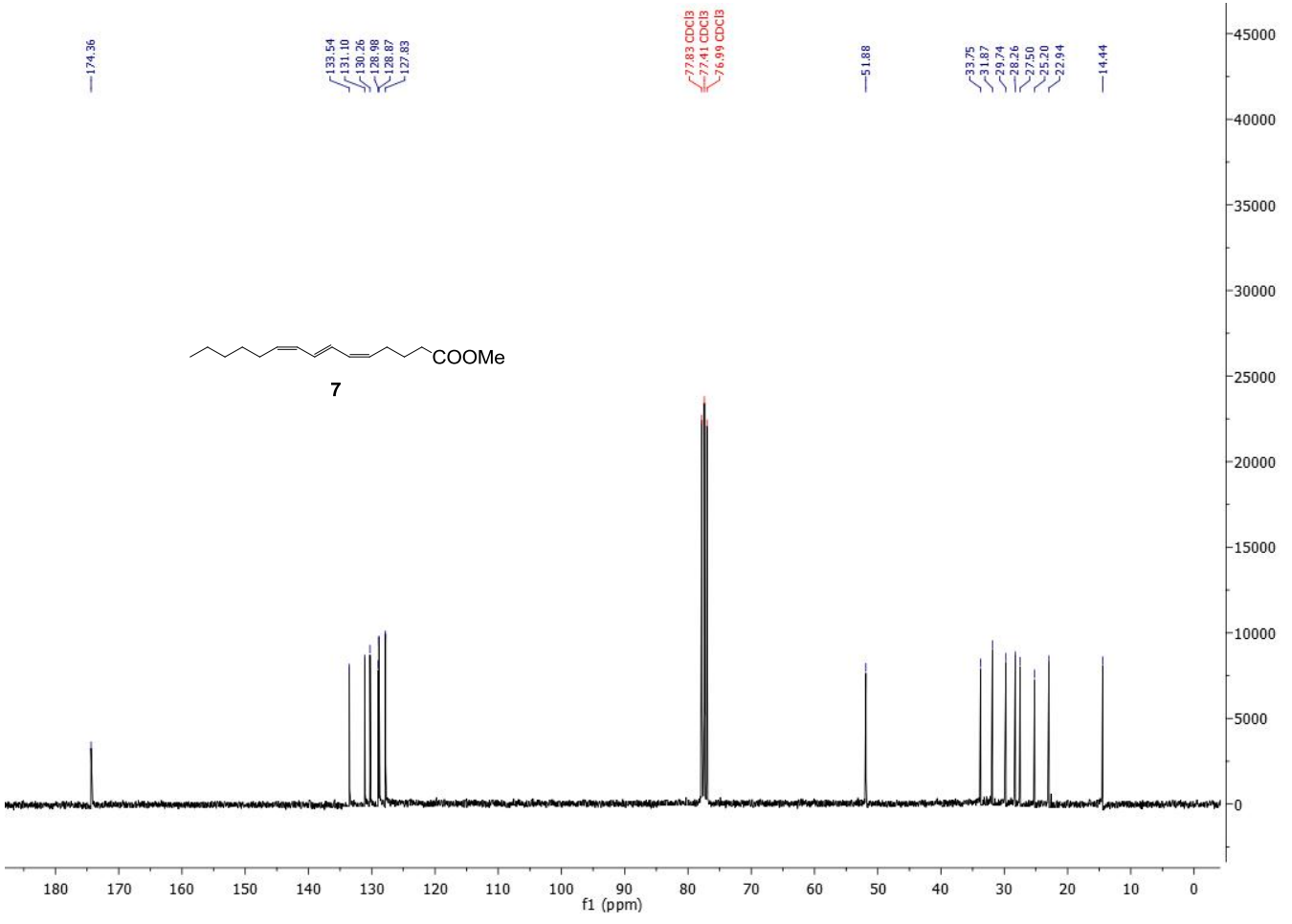
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127.83
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33.75
31.87
29.74
28.26
27.50
25.20
22.94
14.44
COOMe
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40000
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30000
25000
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15000
10000
5000
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f1 (ppm)

Data File D:\DATA\YASSER\0000000000007.D

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                                                  Inj Volume : 15 µl
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Analysis Method : D:\METHODS\Yasser\YM-10 13 min-Alex.m
Last changed     : 4/2/2012 10:44:08 AM by Yasser
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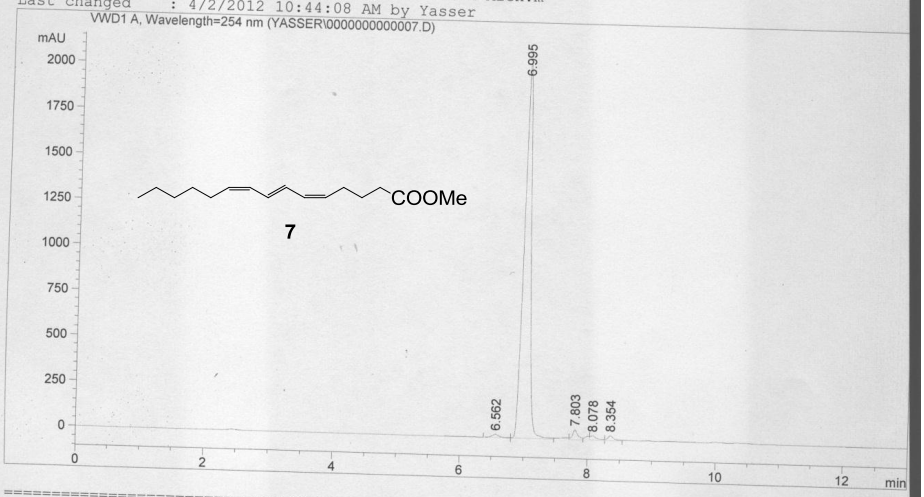


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                        Area Percent Report

Sorted By              :      Signal
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Dilution               :      1.0000
Sample Amount          :      1.00000  [ng/ul]    (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=254 nm

Peak RetTime Type  Width     Area       Height      Area
  #   [min]        [min]  mAU   *s    [mAU   ]       %
----|-------|----|-------|----------|----------|--------|
   1   6.562 VV    0.1154  156.04616   18.87604   0.9492
   2   6.995 VV    0.1133 1.55921e4  2054.67139  94.8398
   3   7.803 VV    0.0866  323.82294   53.54426   1.9697
   4   8.078 VV    0.1024  180.52550   24.37546   1.0981
   5   8.354 VV    0.0992  187.96710   27.31306   1.1433

Totals :                  1.64405e4  2178.78021

                        *** End of Report ***


nstrument 1 4/2/2012 10:45:05 AM Yasser
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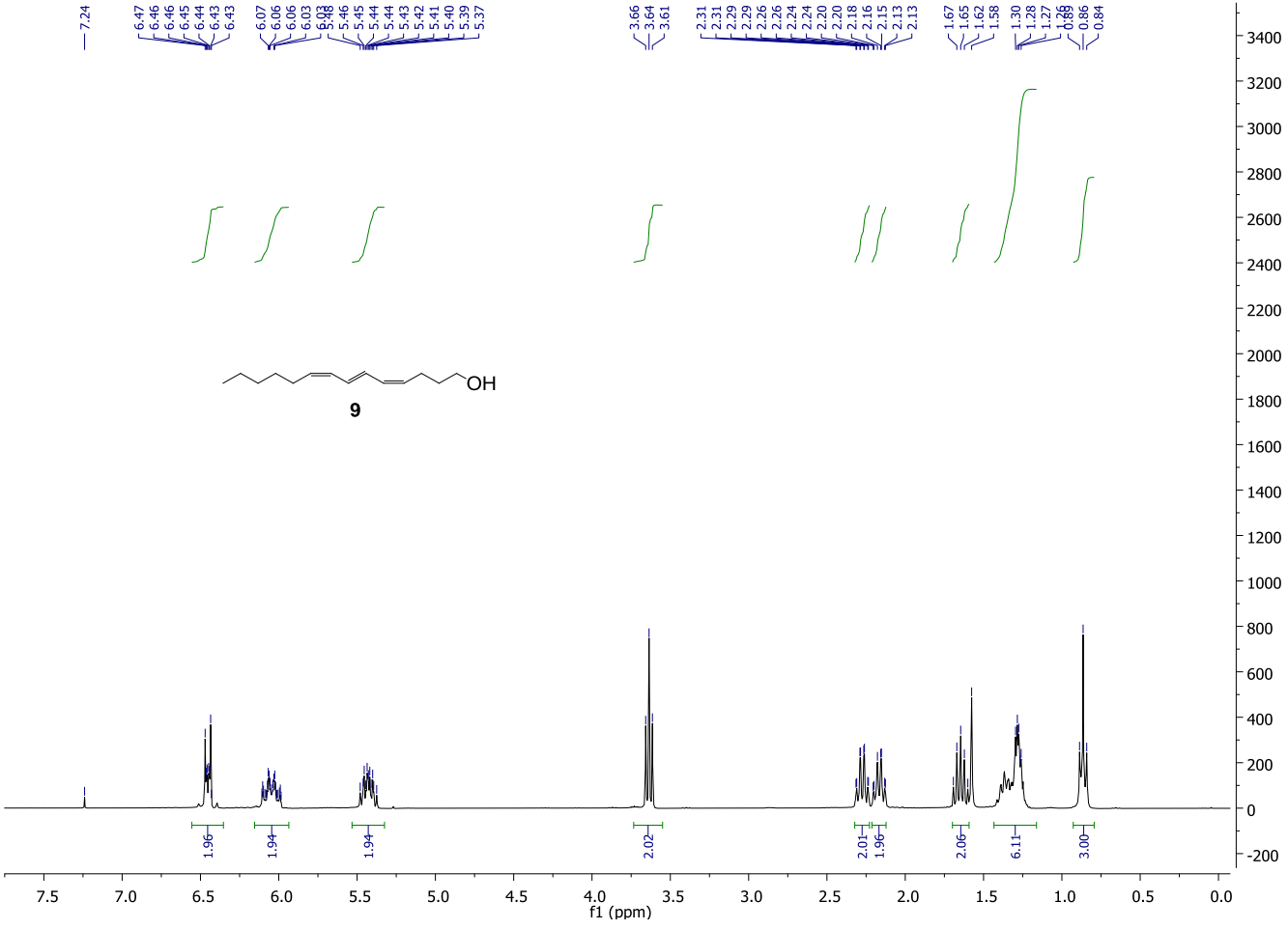
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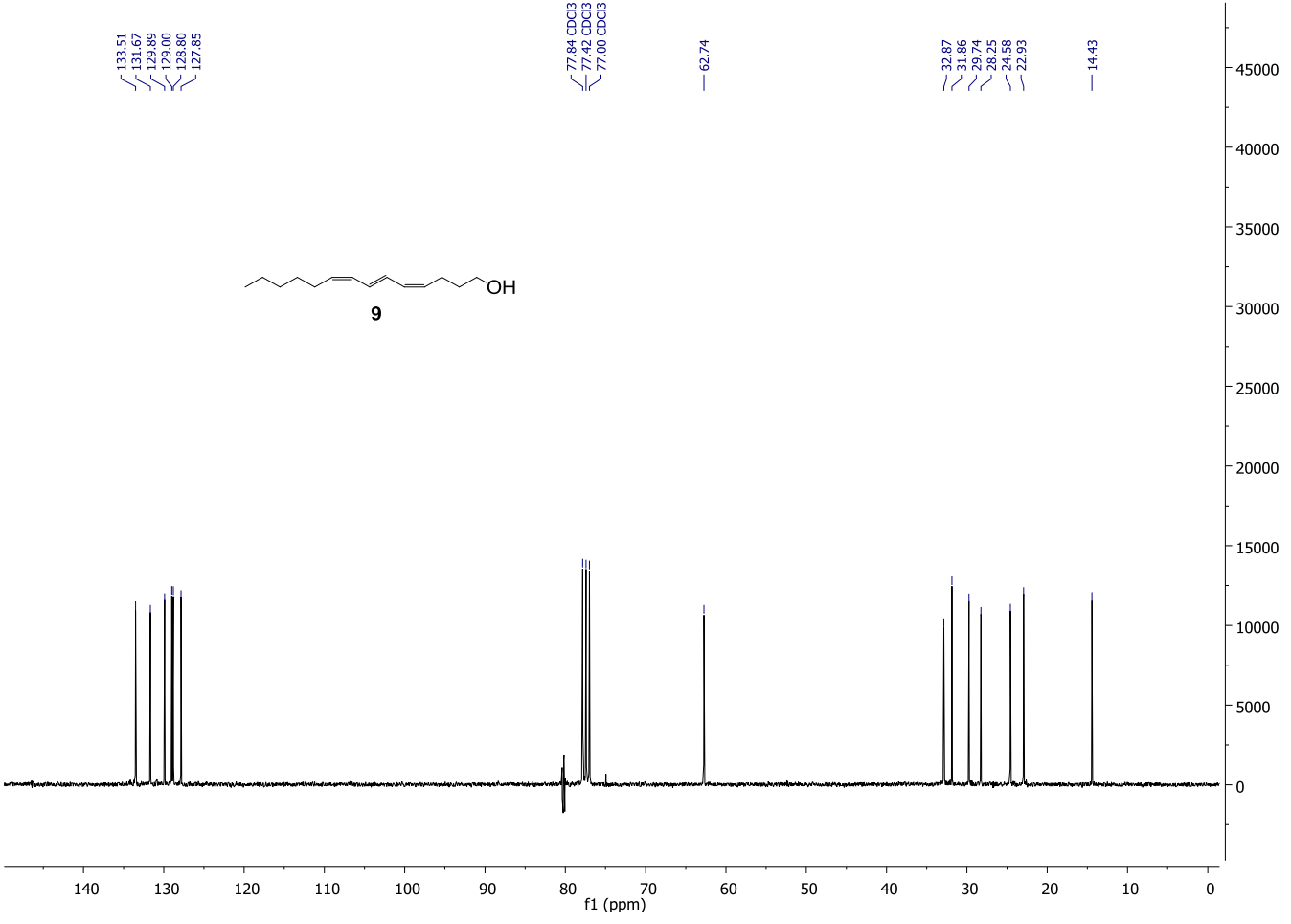
3.00
f1 (ppm)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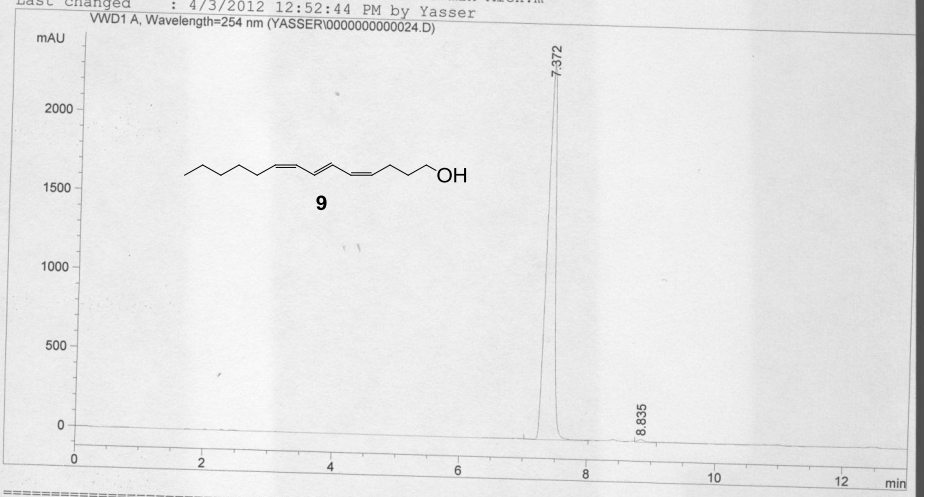

Area Percent Report

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Dilution : 1.0000
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Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=254 nm

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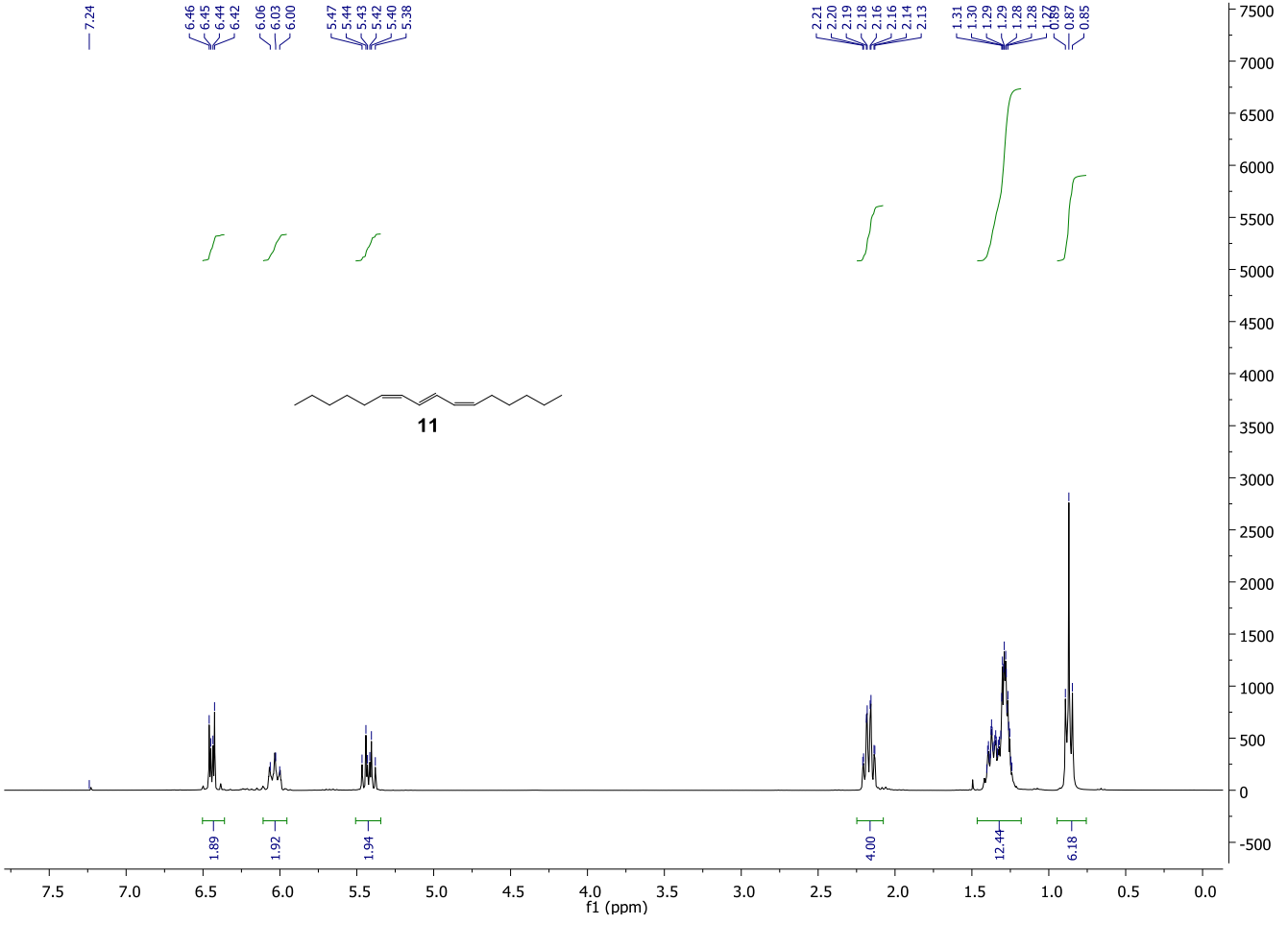
2	8.835	VV	0.1163	150.48865	18.03929	0.7056
Totals :				2.13265e4	2392.39598	

*** End of Report ***

Instrument 1 4/3/2012 12:59:39 PM Yasser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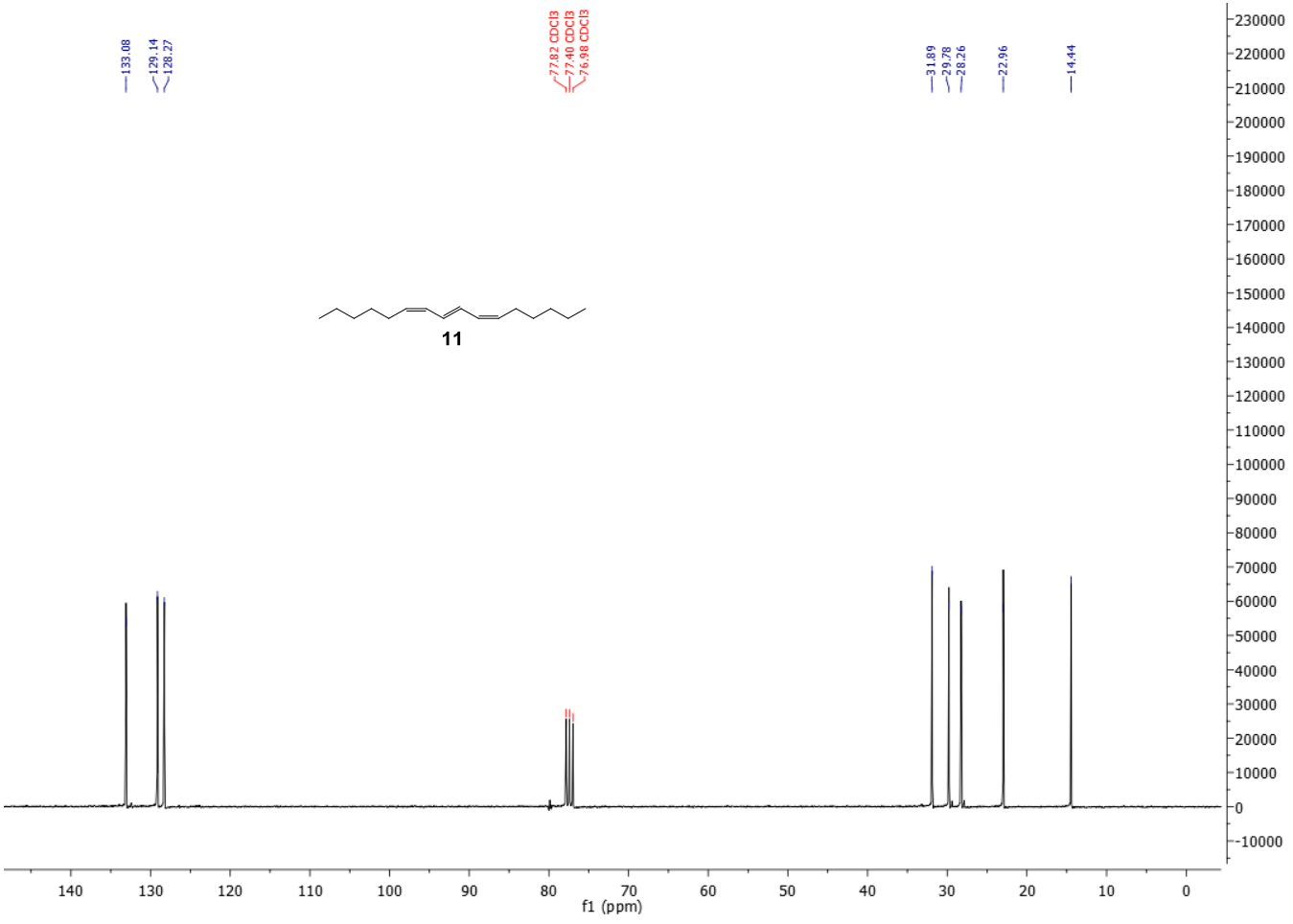
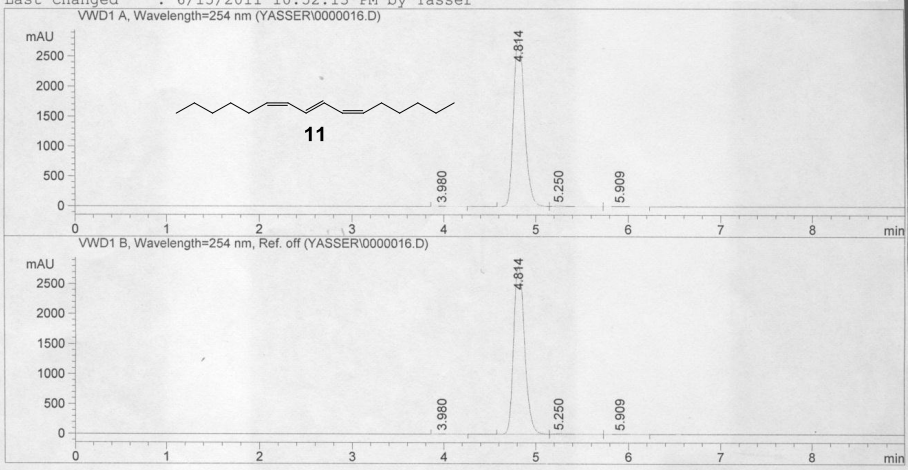
5.40
5.38
2.21
2.20
2.19
2.18
2.16
2.16
2.14
2.13
1.31
1.30
1.29
1.29
1.28
1.28
1.27
0.89
0.87
0.85
11
1.89
1.92
1.94
4.00
12.44
6.18
f1 (ppm)


133.08
129.14
128.27
77.82 CDCl3
77.40 CDCl3
76.98 CDCl3
31.89
29.78
28.26
22.96
14.44
11
f1 (ppm)

Data File D:\DATA\YASSER\0000016.D

Acq. Operator : Yasser
Acq. Instrument : Instrument 1 Location : Vial 1
Injection Date : 6/15/2011 10:42:59 PM
Inj Volume : 2
Acq. Method : D:\METHODS\Yasser\15.06.2011-1.m
Last changed : 6/15/2011 10:44:00 PM by Yasser
Analysis Method : D:\METHODS\Yasser\15.06.2011-1.m
Last changed : 6/15/2011 10:52:13 PM by Yasser



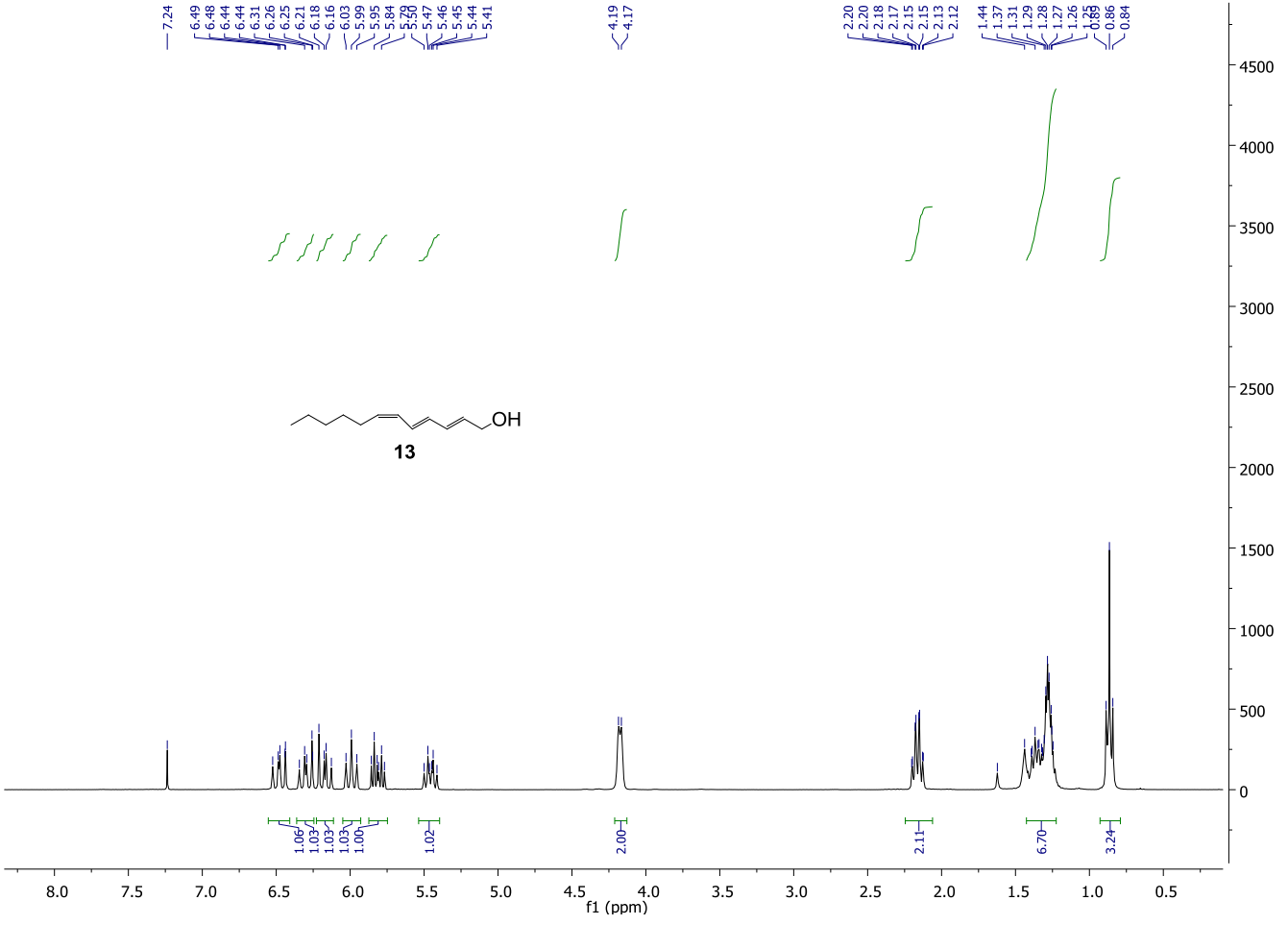
Area Percent Report

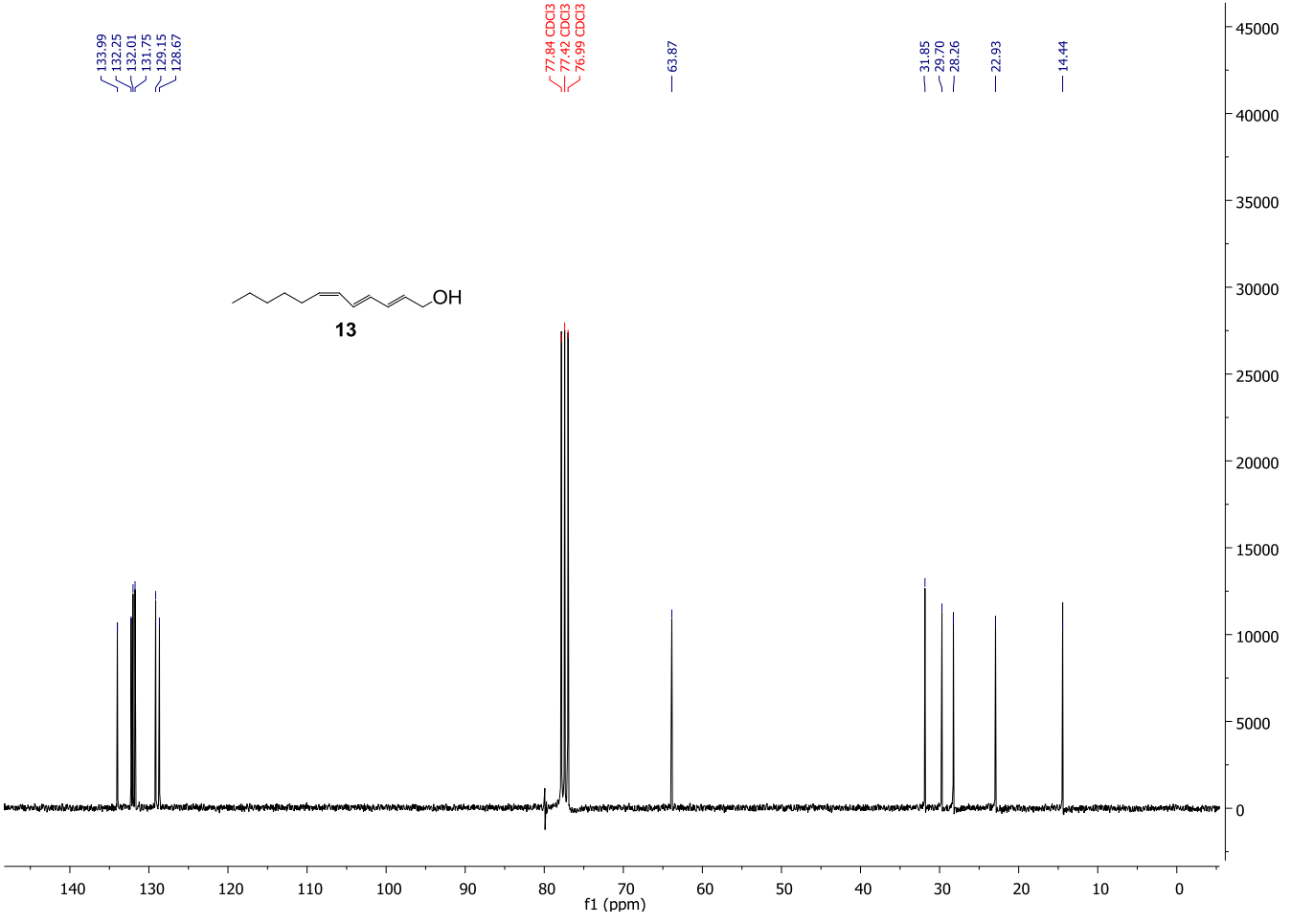
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Sample Amount : 1.00000 [ng/ul] (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	3.980	BB	0.1153	15.33735	1.91522	0.0653
2	4.814	BV	0.1306	2.33387e4	2803.00757	99.3699
3	5.250	VV	0.1525	94.79829	8.86162	0.4036
4	5.909	VB	0.1547	37.85872	3.59952	0.1612
Totals :				2.34867e4	2817.38393	

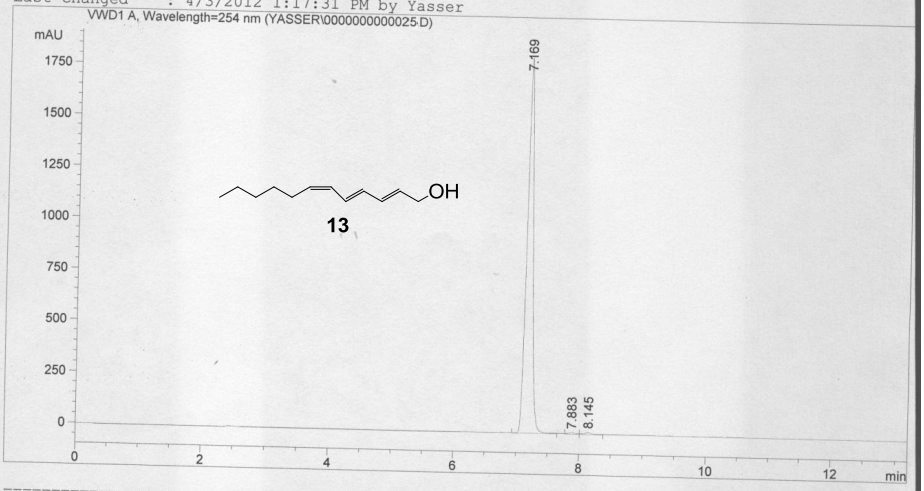
Instrument 1 6/15/2011 10:52:24 PM Yasser


7.24
6.49
6.48
6.44
6.44
6.31
6.26
6.25
6.21
6.18
6.16
6.03
5.99
5.95
5.84
5.50
5.47
5.46
5.45
5.44
5.41
4.19
4.17
2.20
2.20
2.18
2.17
2.15
2.15
2.13
2.12
1.44
1.37
1.31
1.29
1.28
1.27
1.26
0.89
0.86
0.84
OH
13
1.06
1.03
1.03
1.03
1.00
1.02
2.00
2.11
6.70
3.24
f1 (ppm)


133.99
132.25
132.01
131.75
129.15
128.67
77.84 CDCl3
77.42 CDCl3
76.99 CDCl3
63.87
31.85
29.70
28.26
22.93
14.44
OH
13
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

Data File D:\DATA\YASSER\0000000000025.D

```
Acq. Operator   : Yasser
Acq. Instrument : Instrument 1                    Location : Vial 1
Injection Date  : 4/3/2012 11:23:50 PM

                                                  Inj Volume : 15 µl
Acq. Method     : D:\METHODS\Cezarina\Gradient 13 min LCMS.m
Last changed    : 4/3/2012 11:00:05 PM by Yasser
Analysis Method : D:\METHODS\Cezarina\Gradient 13 min LCMS.m
Last changed    : 4/3/2012 1:17:31 PM by Yasser
```



Area Percent Report

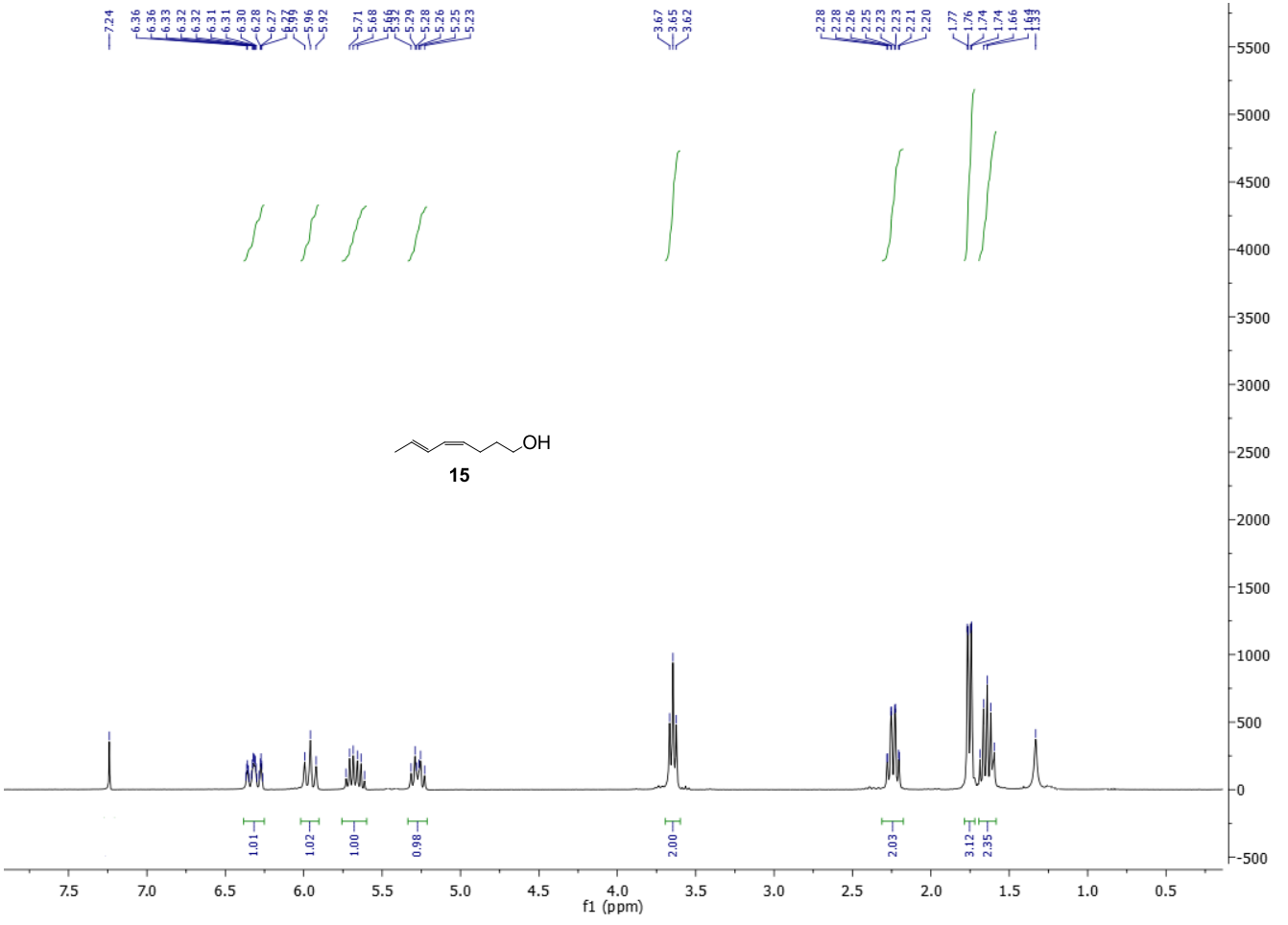
```
Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount         :      1.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs
```

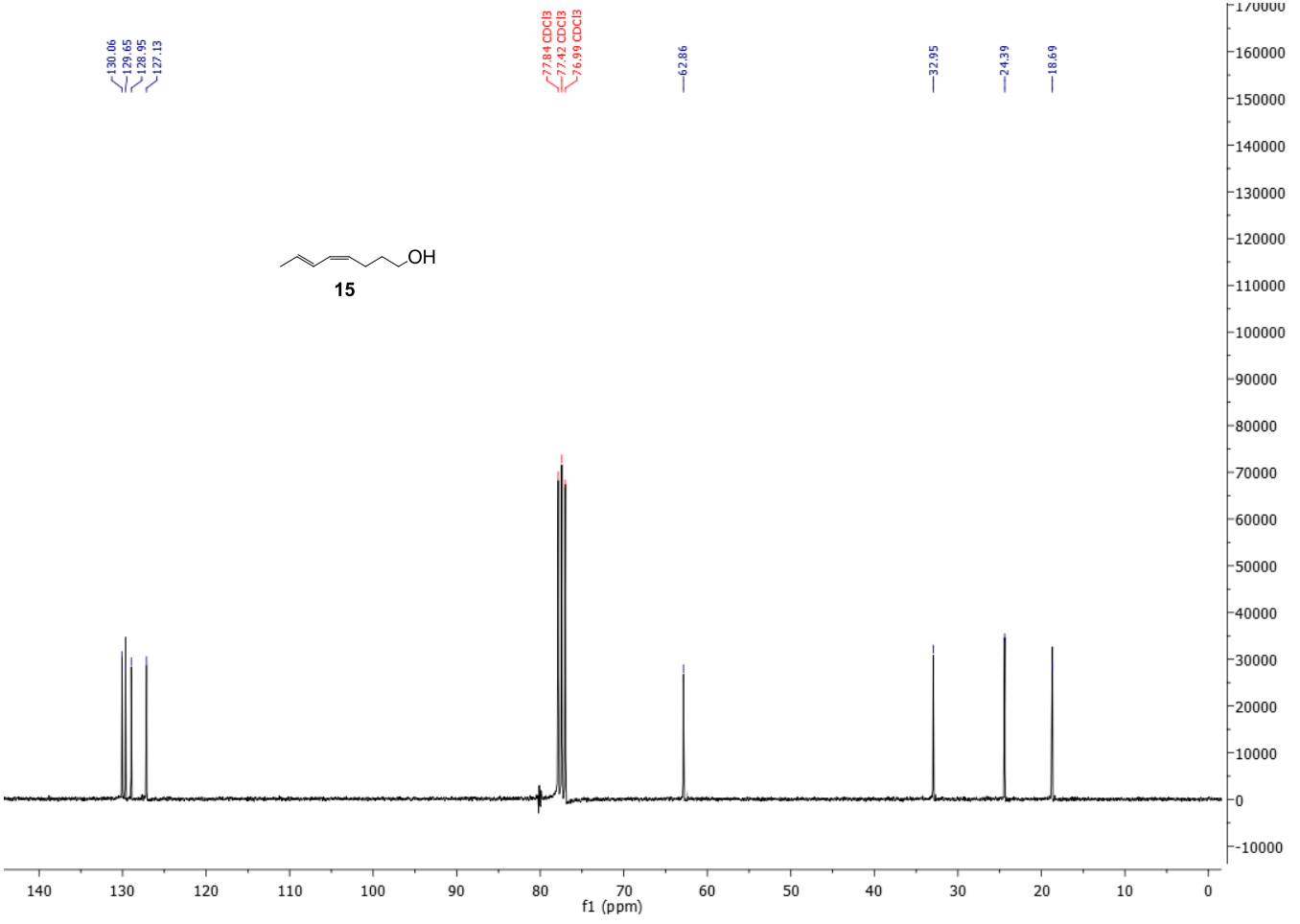
Signal 1: VWD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	7.169	VV	0.1026	1.23918e4	1822.44189	98.6784
2	7.883	VV	0.1022	66.24044	8.97125	0.5275
3	8.145	VV	0.1219	99.72744	11.14329	0.7942
Totals :				1.25577e4	1842.55644	

*** End of Report ***

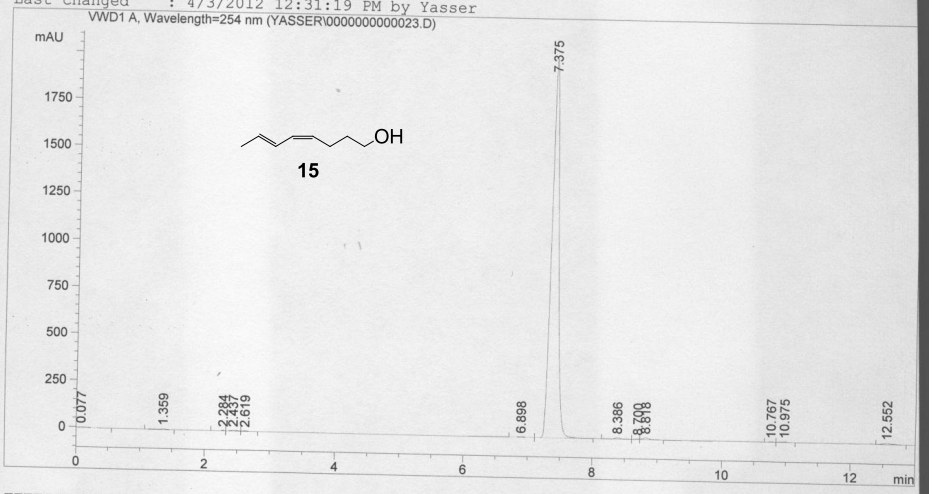
nstrument 1 4/3/2012 1:18:07 PM Yasser


7.24
6.36
6.36
6.33
6.32
6.32
6.31
6.31
6.30
6.28
6.27
5.96
5.92
5.71
5.68
5.32
5.29
5.28
5.26
5.25
5.23
3.67
3.65
3.62
2.28
2.28
2.26
2.25
2.23
2.23
2.21
2.20
1.77
1.76
1.74
1.74
1.66
OH
15
1.01
1.02
1.00
0.98
2.00
2.03
3.12
2.35
f1 (ppm)


130.06
129.65
128.95
127.13
77.84 CDCl3
77.42 CDCl3
76.99 CDCl3
62.86
32.95
24.39
18.69
OH
15
f1 (ppm)

Data File D:\DATA\YASSER\0000000000023.D

```
Acq. Operator   : Yasser
Acq. Instrument : Instrument 1                    Location : Vial 2
Injection Date  : 4/3/2012 12:17:58 PM

                                                  Inj Volume : 15 µl
Acq. Method     : D:\METHODS\Yasser\YM-10 13 min-Alex.m
Last changed    : 4/3/2012 12:17:06 PM by Yasser
Analysis Method : D:\METHODS\Yasser\YM-10 13 min-Alex.m
Last changed    : 4/3/2012 12:31:19 PM by Yasser
```



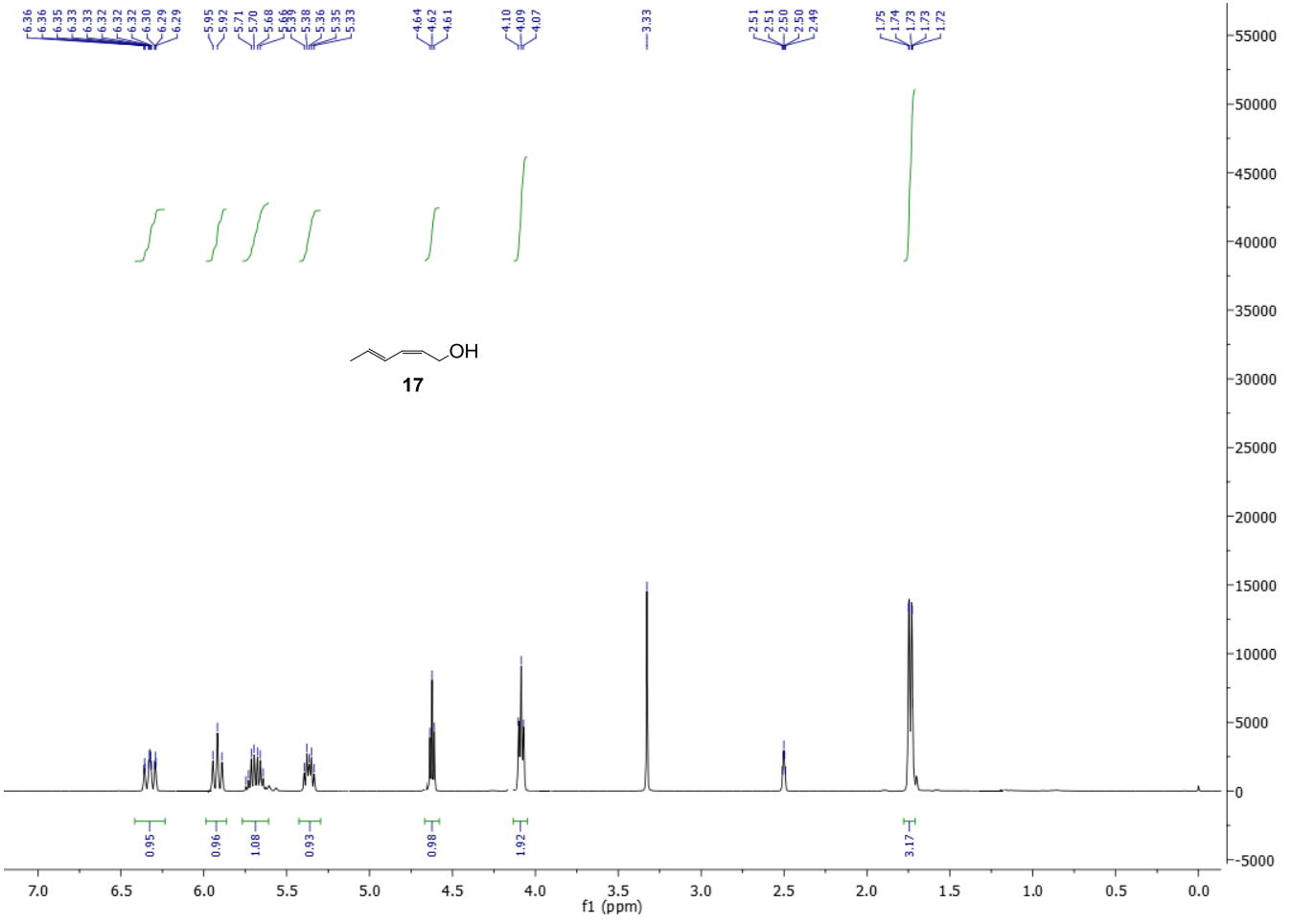
Area Percent Report

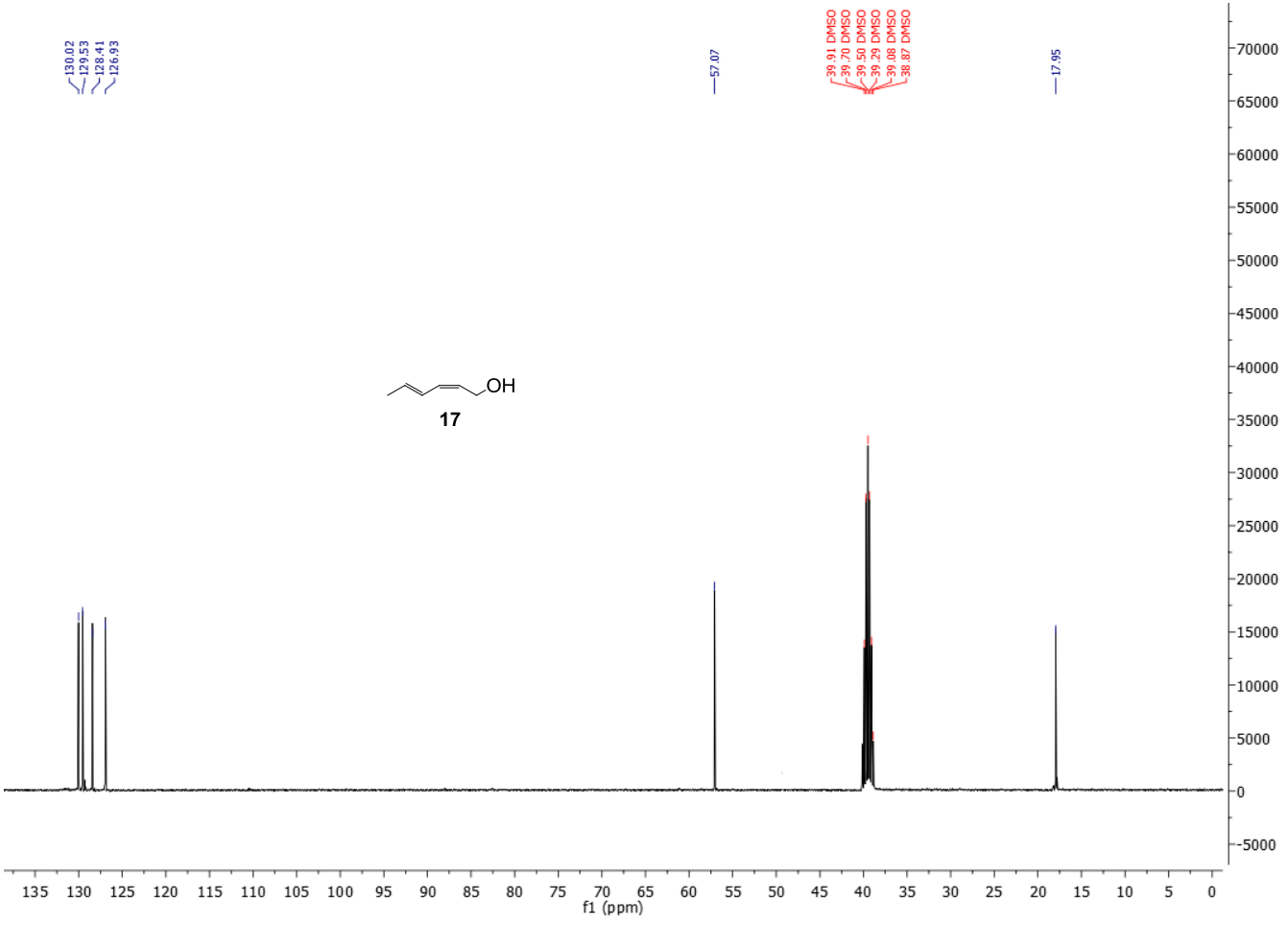
```
Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount         :      1.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs
```

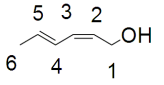
Signal 1: VWD1 A, Wavelength=254 nm

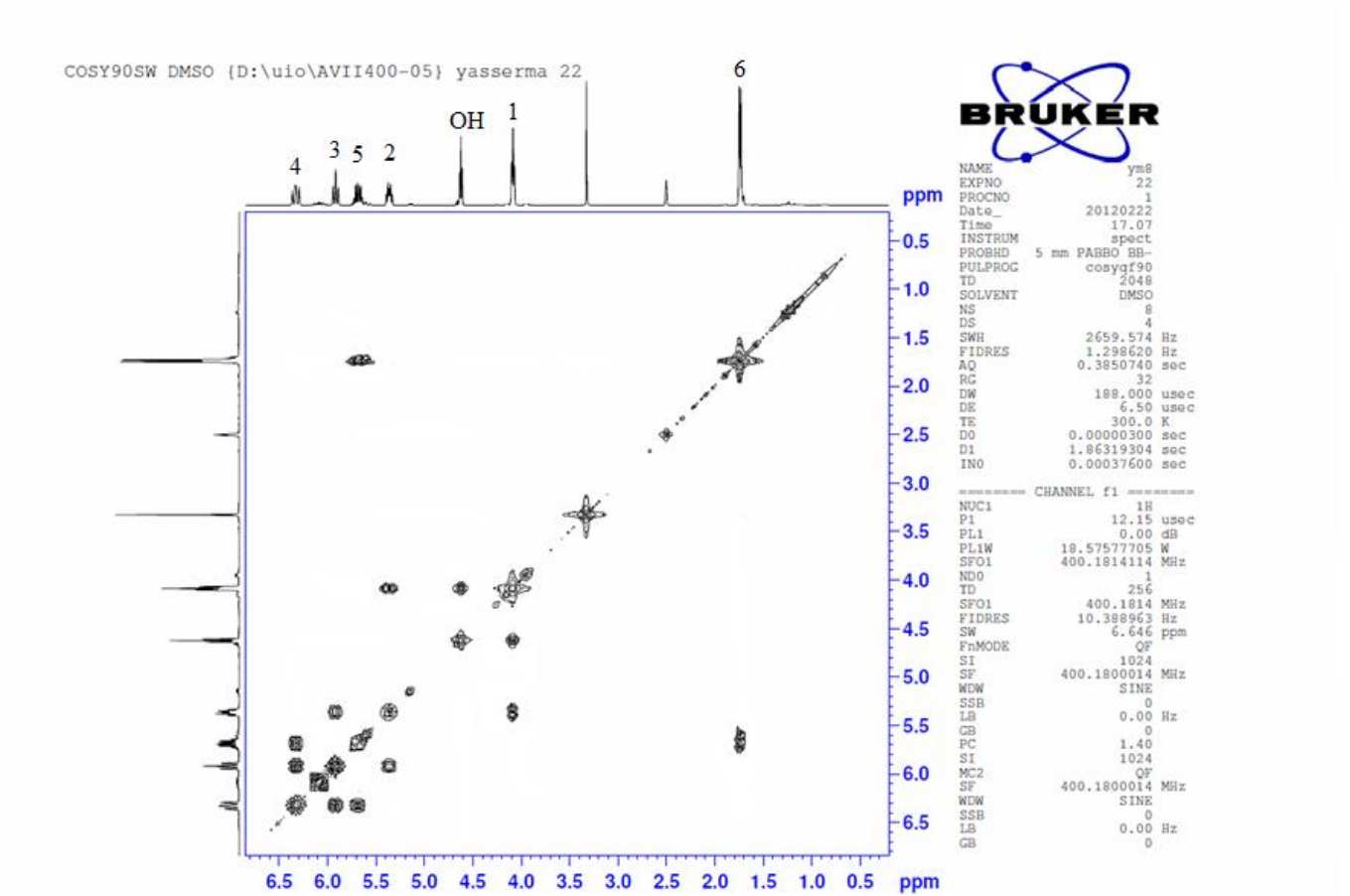
Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	0.077	BB	0.1646	24.41685	2.57267	0.1531
2	1.359	BB	0.0849	17.35588	3.00371	0.1088
3	2.284	VV	0.0595	9.77310	2.31325	0.0613
4	2.437	VV	0.1039	27.51729	3.84370	0.1726
5	2.619	VB	0.0918	12.34903	1.90063	0.0774
6	6.898	VV	0.1203	15.86633	1.85343	0.0995
7	7.375	VV	0.1165	1.55828e4	2012.80188	97.7176
8	8.386	VV	0.1246	67.80808	7.38434	0.4252
9	8.700	VV	0.0810	15.85873	2.91447	0.0994
10	8.818	VB	0.1005	75.03494	10.72754	0.4705
11	10.767	VV	0.0990	14.68287	2.17974	0.0921
12	10.975	VV	0.1244	26.02958	2.92091	0.1632
13	12.552	VV	0.1856	57.27394	4.61241	0.3592

nstrument 1 4/3/2012 12:31:30 PM Yasser


6.36
6.36
6.35
6.33
6.33
6.32
6.32
6.32
6.30
6.29
6.29
5.95
5.92
5.71
5.70
5.68
5.66
5.38
5.36
5.35
5.33
4.64
4.62
4.61
4.10
4.09
4.07
3.33
2.51
2.51
2.50
2.50
2.49
1.75
1.74
1.73
1.73
1.72
OH
17
55000
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
-5000
0.95
0.96
1.08
0.93
0.98
1.92
3.17
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


130.02
129.53
128.41
126.93
57.07
39.91 DMSO
39.70 DMSO
39.50 DMSO
39.29 DMSO
39.08 DMSO
38.87 DMSO
17.95
OH
17
f1 (ppm)

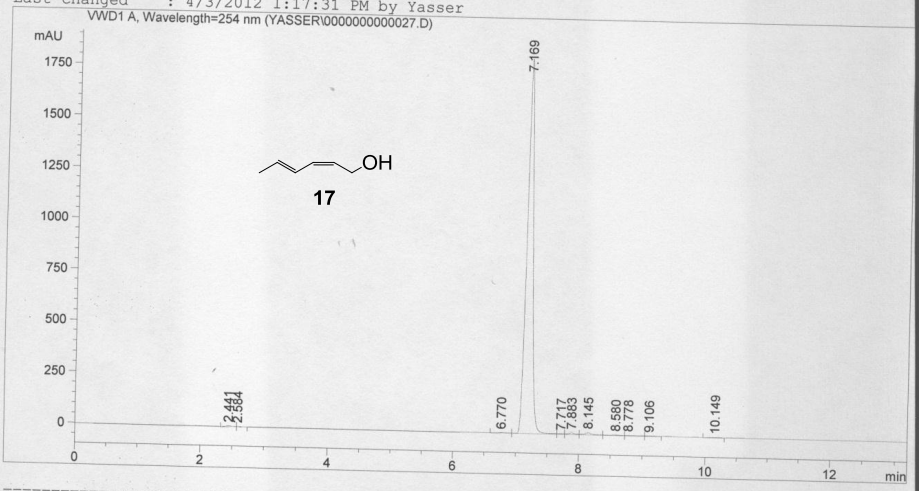

5 3 2
OH
6 4 1


COSY90SW DMSO {D:\uio\AVII400-05} yasserma 22
4
3
5
2
OH
1
6
ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
BRUKER
NAME ym8
EXPNO 22
PROCNO 1
Date_ 20120222
Time 17.07
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG cosygf90
TD 2048
SOLVENT DMSO
NS 8
DS 4
SWH 2659.574 Hz
FIDRES 1.298620 Hz
AQ 0.3850740 sec
RG 32
DW 188.000 usec
DE 6.50 usec
TE 300.0 K
D0 0.00000300 sec
D1 1.86319304 sec
IN0 0.00037600 sec
======== CHANNEL f1 ========
NUC1 1H
P1 12.15 usec
PL1 0.00 dB
PL1W 18.57577705 W
SFO1 400.1814114 MHz
ND0 1
TD 256
SFO1 400.1814 MHz
FIDRES 10.388963 Hz
SW 6.646 ppm
FnMODE QF
SI 1024
SF 400.1800014 MHz
WDW SINE
SSB 0
LB 0.00 Hz
GB 0
PC 1.40
SI 1024
MC2 QF
SF 400.1800014 MHz
WDW SINE
SSB 0
LB 0.00 Hz
GB 0

Data File D:\DATA\YASSER\0000000000027.D

Acq. Operator : Yasser
Acq. Instrument : Instrument 1 Location : Vial 4
Injection Date : 4/3/2012 1:03:59 PM Inj Volume : 15 µl

Acq. Method : D:\METHODS\Cezarina\Gradient 13 min LCMS.m
Last changed : 4/3/2012 1:03:05 PM by Yasser
Analysis Method : D:\METHODS\Cezarina\Gradient 13 min LCMS.m
Last changed : 4/3/2012 1:17:31 PM by Yasser



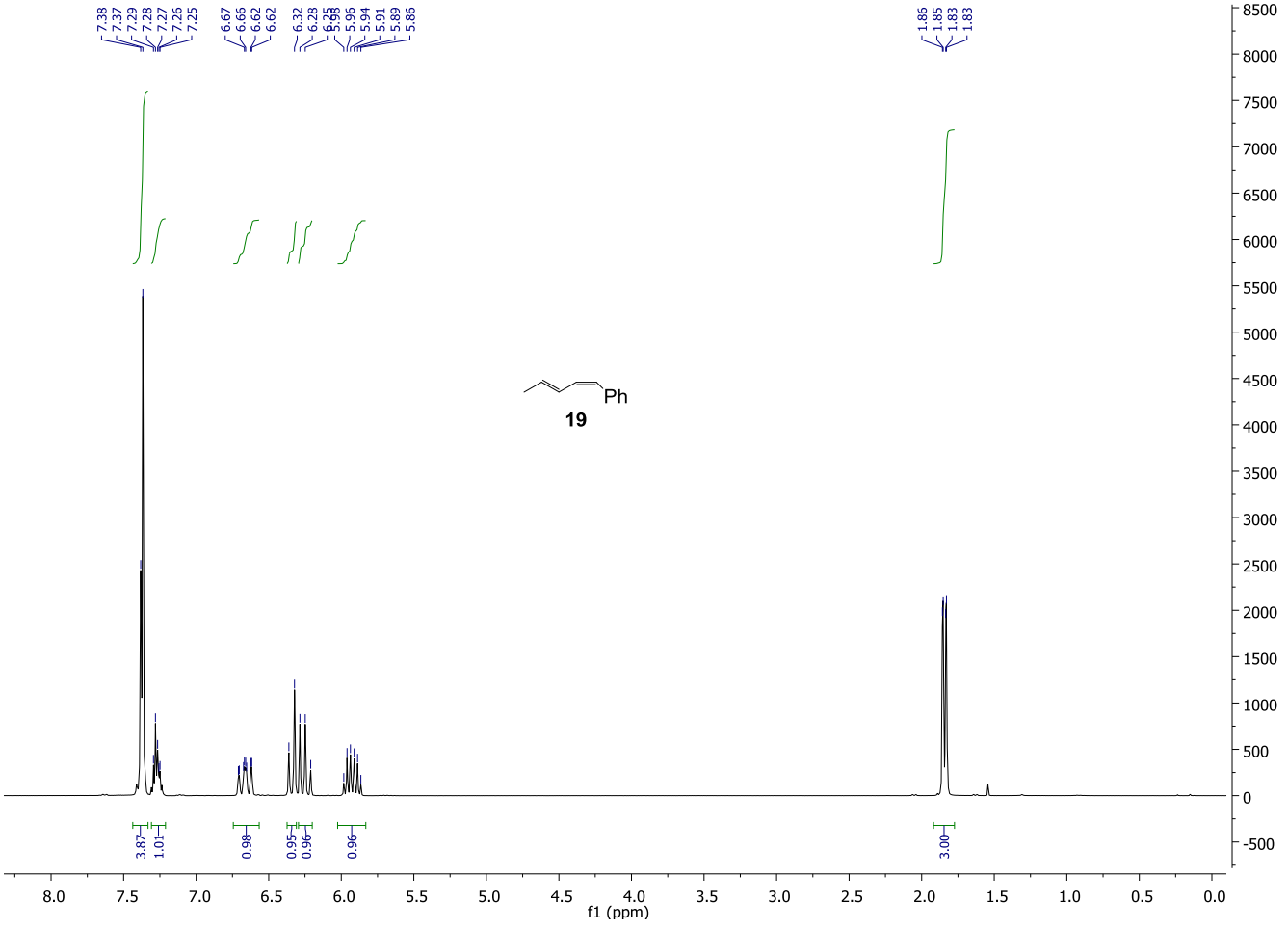
Area Percent Report

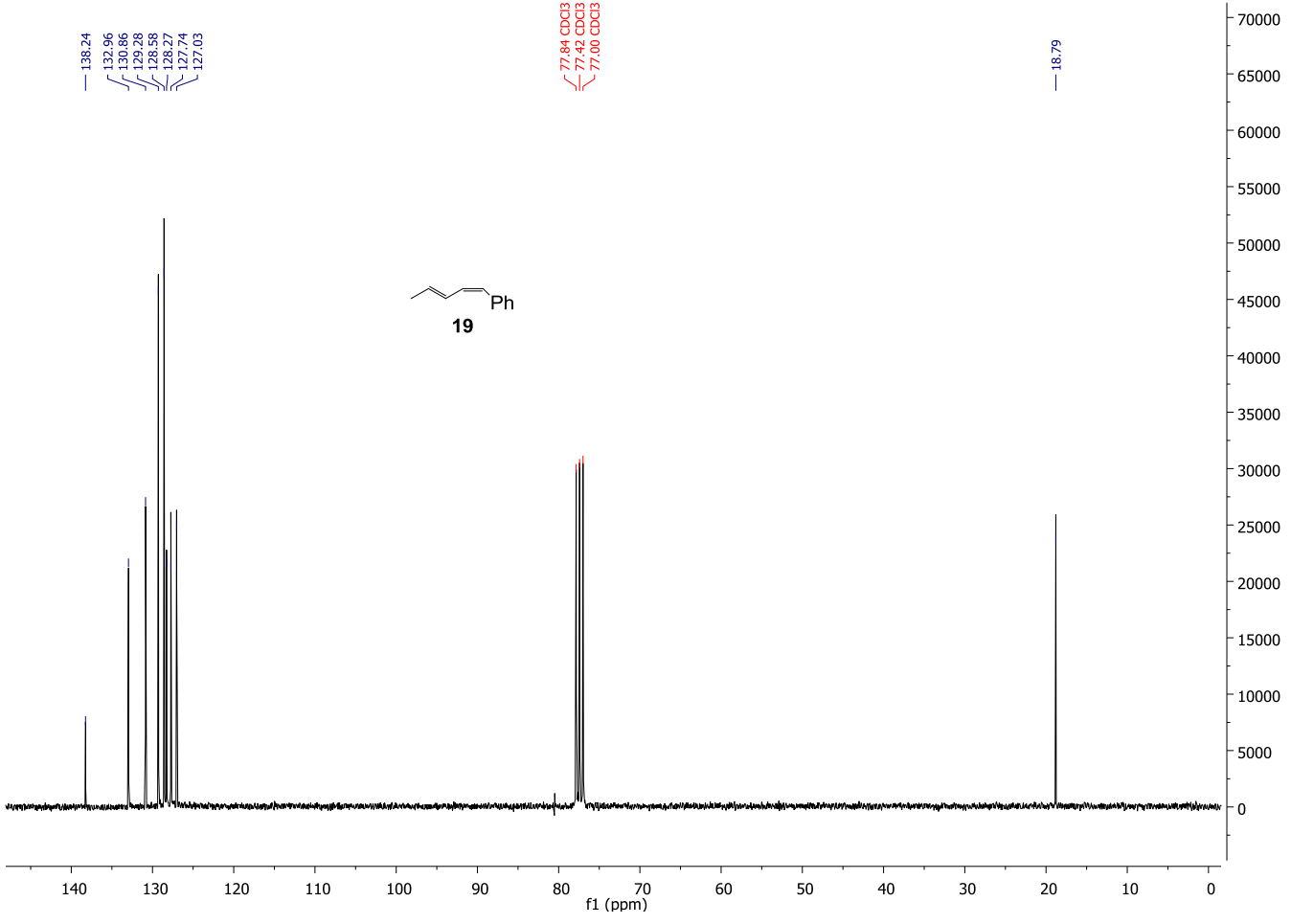
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Sample Amount : 1.00000 [ng/ul] (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	2.441	VV	0.1176	43.68092	5.16504	0.3413
2	2.584	VB	0.0828	10.83421	1.77738	0.0847
3	6.770	VV	0.1506	26.91645	2.38559	0.2103
4	7.169	VV	0.1026	1.23918e4	1822.44189	96.8338
5	7.717	VV	0.0971	21.66357	3.06365	0.1693
6	7.883	VV	0.1022	66.24044	8.97125	0.5176
7	8.145	VV	0.1219	99.72744	11.14329	0.7793
8	8.580	VV	0.2255	47.30481	2.74956	0.3697
9	8.778	VV	0.2101	38.38354	2.32454	0.2999
10	9.106	VV	0.1772	23.74515	1.81749	0.1856
11	10.149	VV	0.1976	26.67916	1.84736	0.2085
Totals :				1.27969e4	1863.68705	

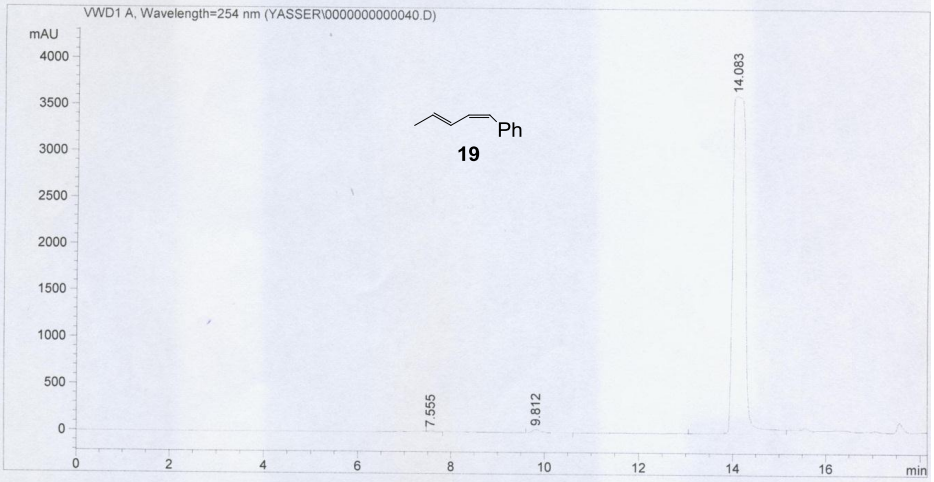
nstrument 1 4/3/2012 1:17:42 PM Yasser


7.38
7.37
7.29
7.28
7.27
7.26
7.25
6.67
6.66
6.62
6.62
6.32
6.28
5.96
5.94
5.91
5.89
5.86
1.86
1.85
1.83
1.83
Ph
19
3.87
1.01
0.98
0.95
0.96
0.96
3.00
f1 (ppm)

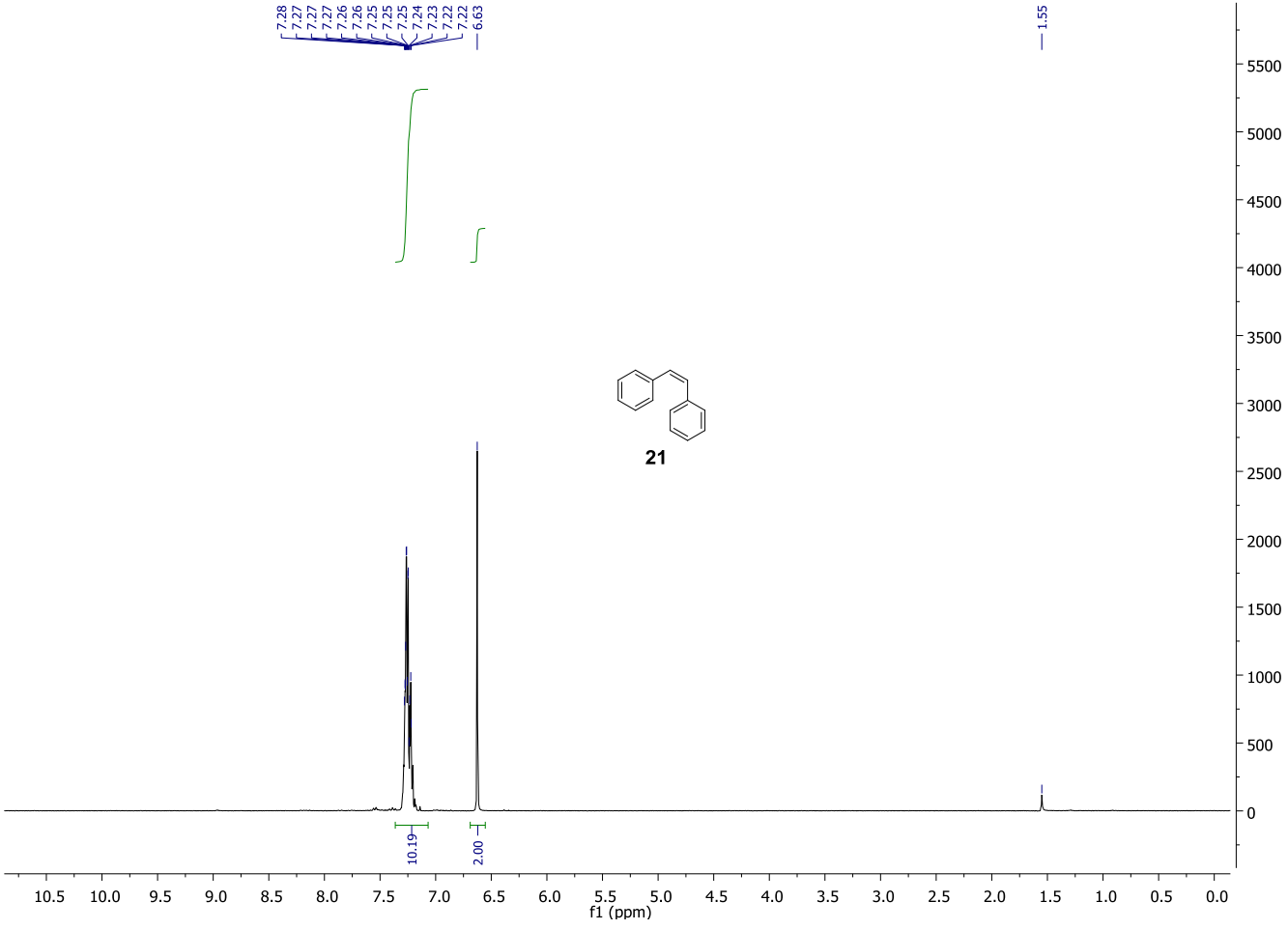

138.24
132.96
130.86
129.28
128.58
128.27
127.74
127.03
77.84 CDCl3
77.42 CDCl3
77.00 CDCl3
18.79
Ph
19
f1 (ppm)

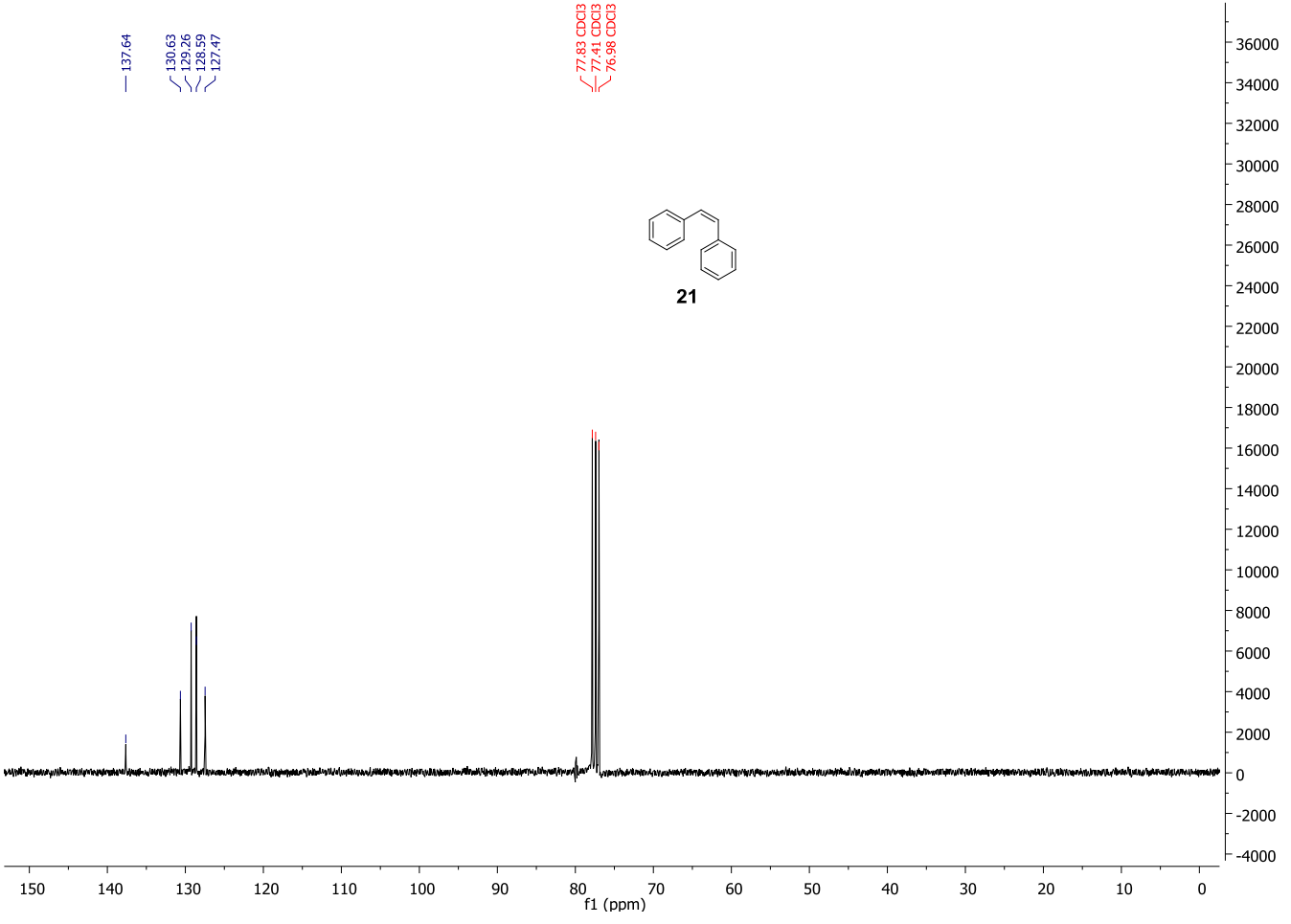
Data File D:\DATA\YASSER\0000000000040.D

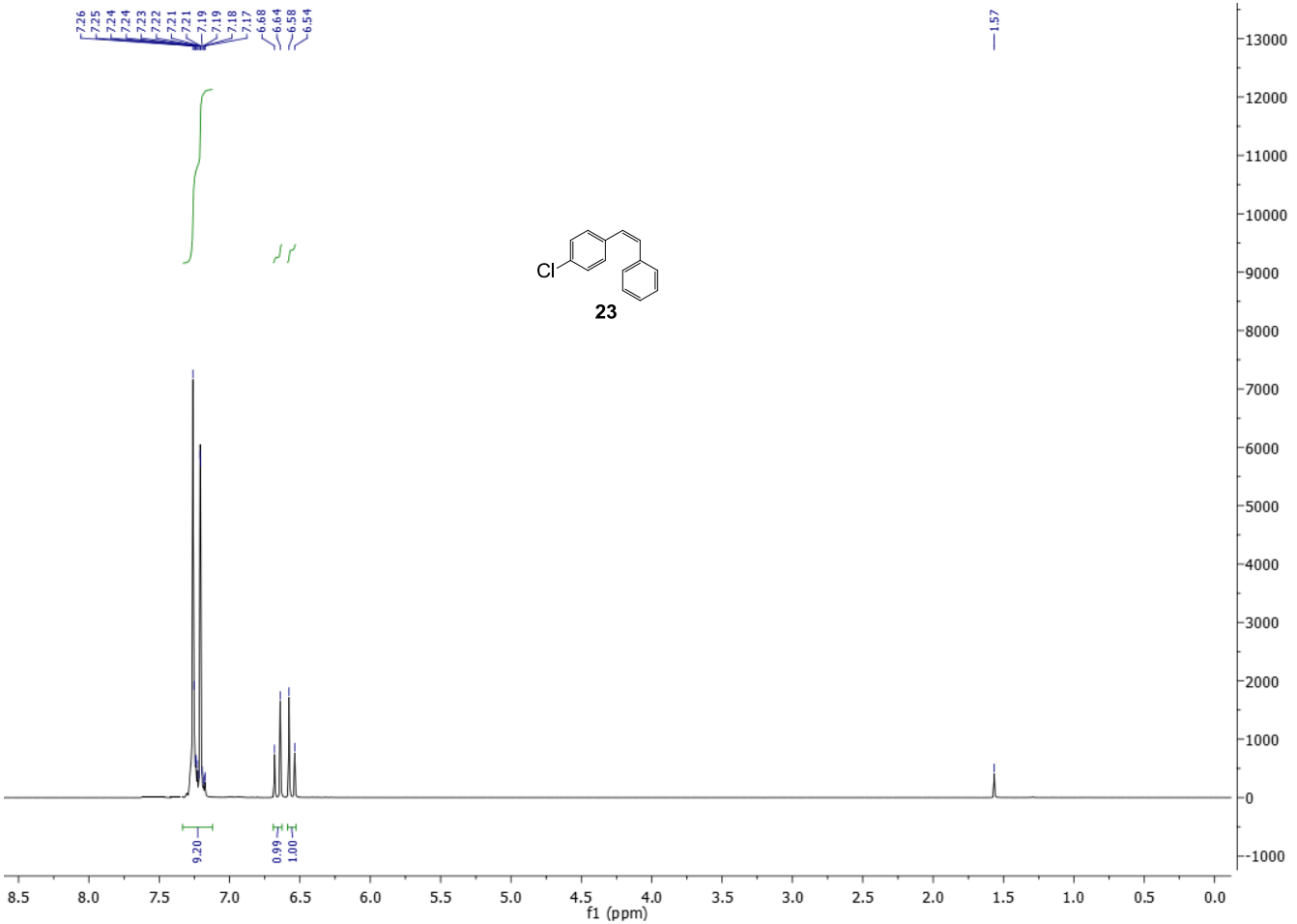
```
Acq. Operator   : Yasser
Acq. Instrument : Instrument 1                    Location : Vial 1
Injection Date  : 9/3/2012 4:11:44 PM
                                                  Inj Volume : 15 µl
Acq. Method     : D:\METHODS\Yasser\YM-10 13 min-Alex.m
Last changed    : 9/3/2012 4:10:59 PM by Yasser
Analysis Method : D:\METHODS\spalare.m
Last changed    : 9/5/2012 2:55:18 PM by Yasser
                :
```

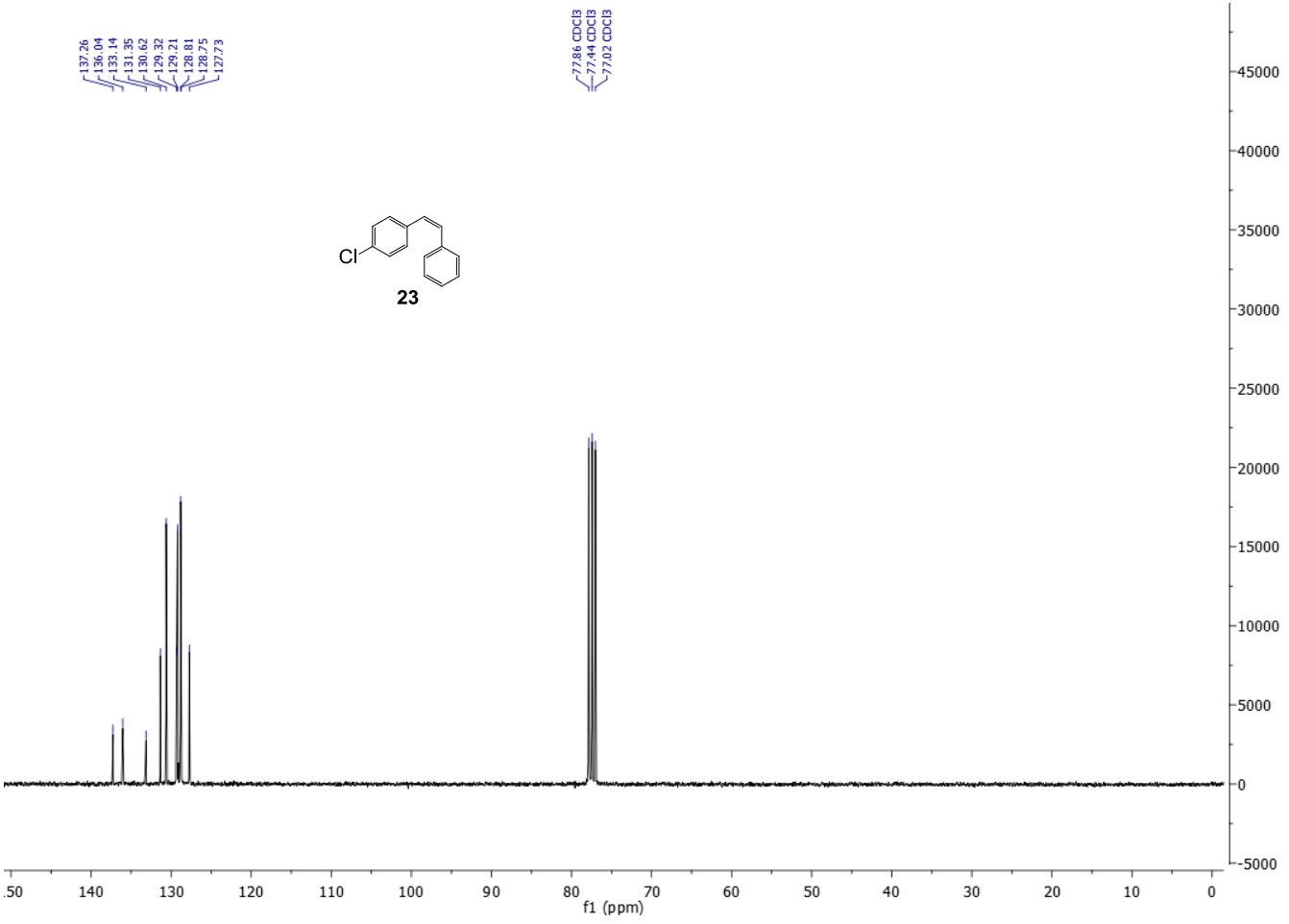


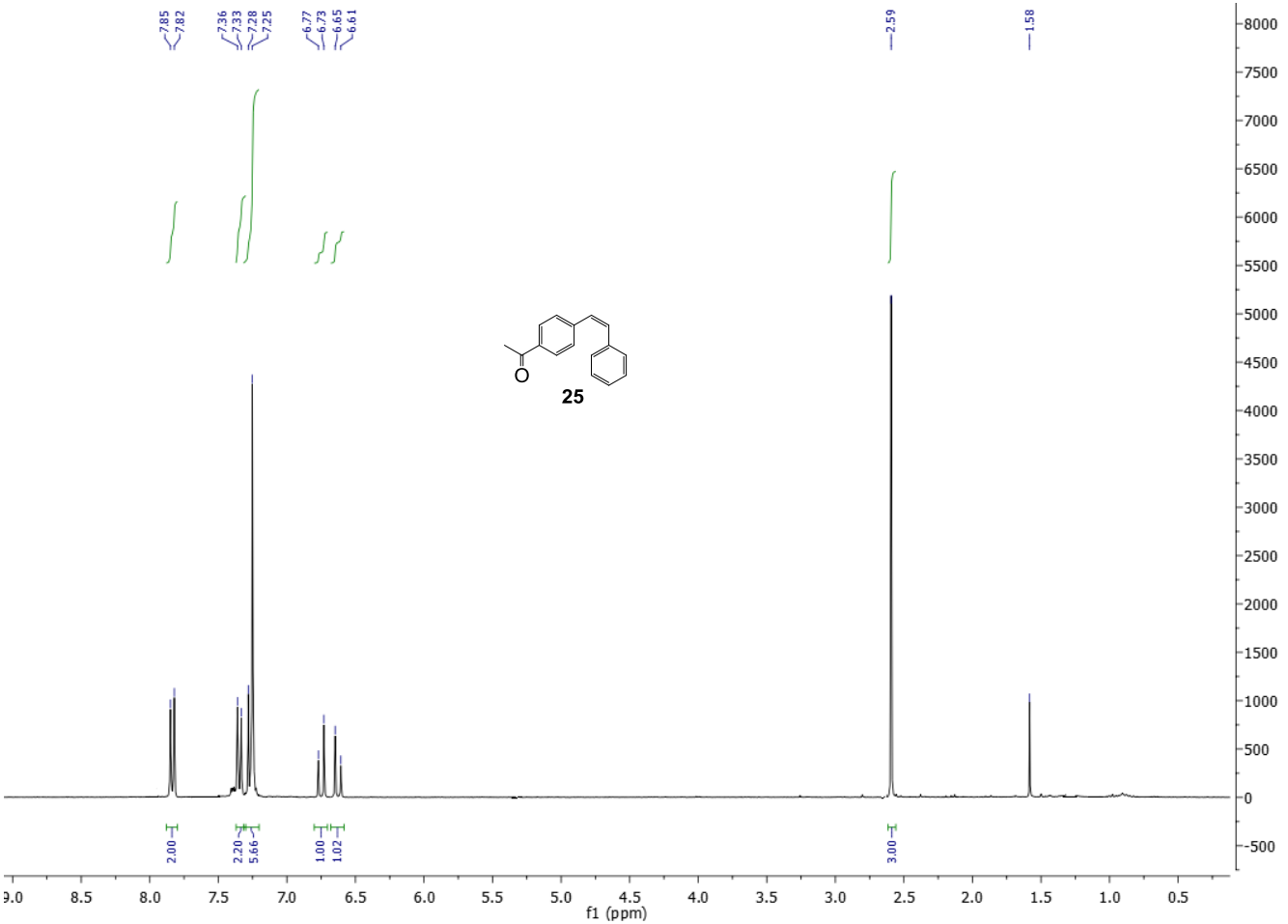

```
                          Area Percent Report

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount         :      1.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=254 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]  mAU   *s   [mAU   ]      %
----|-------|----|-------|----------|----------|--------|
   1   7.555 VV    0.1935  212.60556   14.20495   0.3012
   2   9.812 VB    0.1907  467.67868   35.70211   0.6626
   3  14.083 VV    0.2672 6.99067e4  3625.87988  99.0362

Totals :'               7.05870e4  3675.78695

                          *** End of Report ***

Instrument 1 9/5/2012 2:59:32 PM Yasser
```

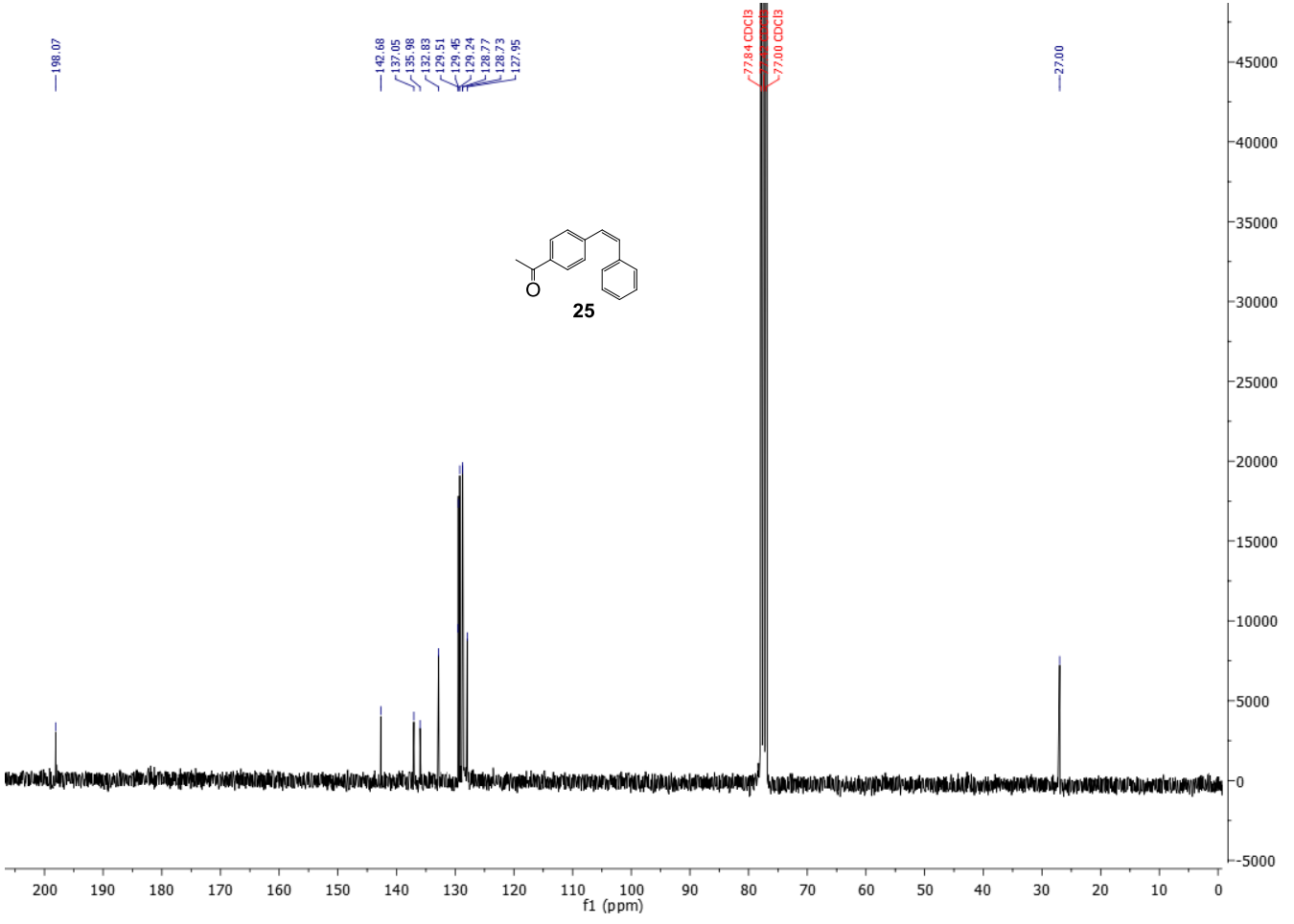

7.28
7.27
7.27
7.27
7.26
7.26
7.25
7.25
7.25
7.24
7.23
7.22
7.22
6.63
1.55
21
10.19
2.00
f1 (ppm)

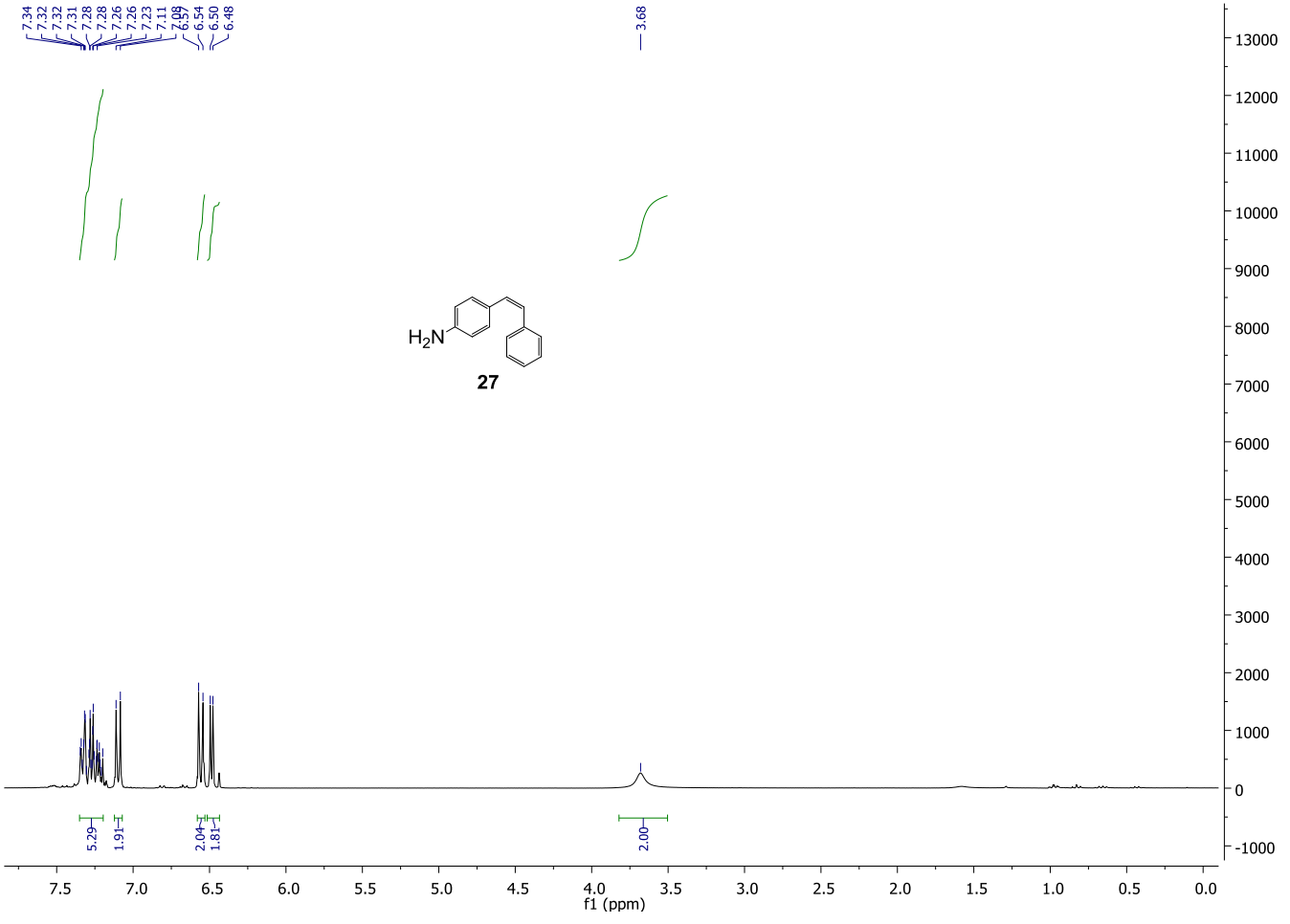

137.64
130.63
129.26
128.59
127.47
77.83 CDCl3
77.41 CDCl3
76.98 CDCl3
21
36000
34000
32000
30000
28000
26000
24000
22000
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
-2000
-4000
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

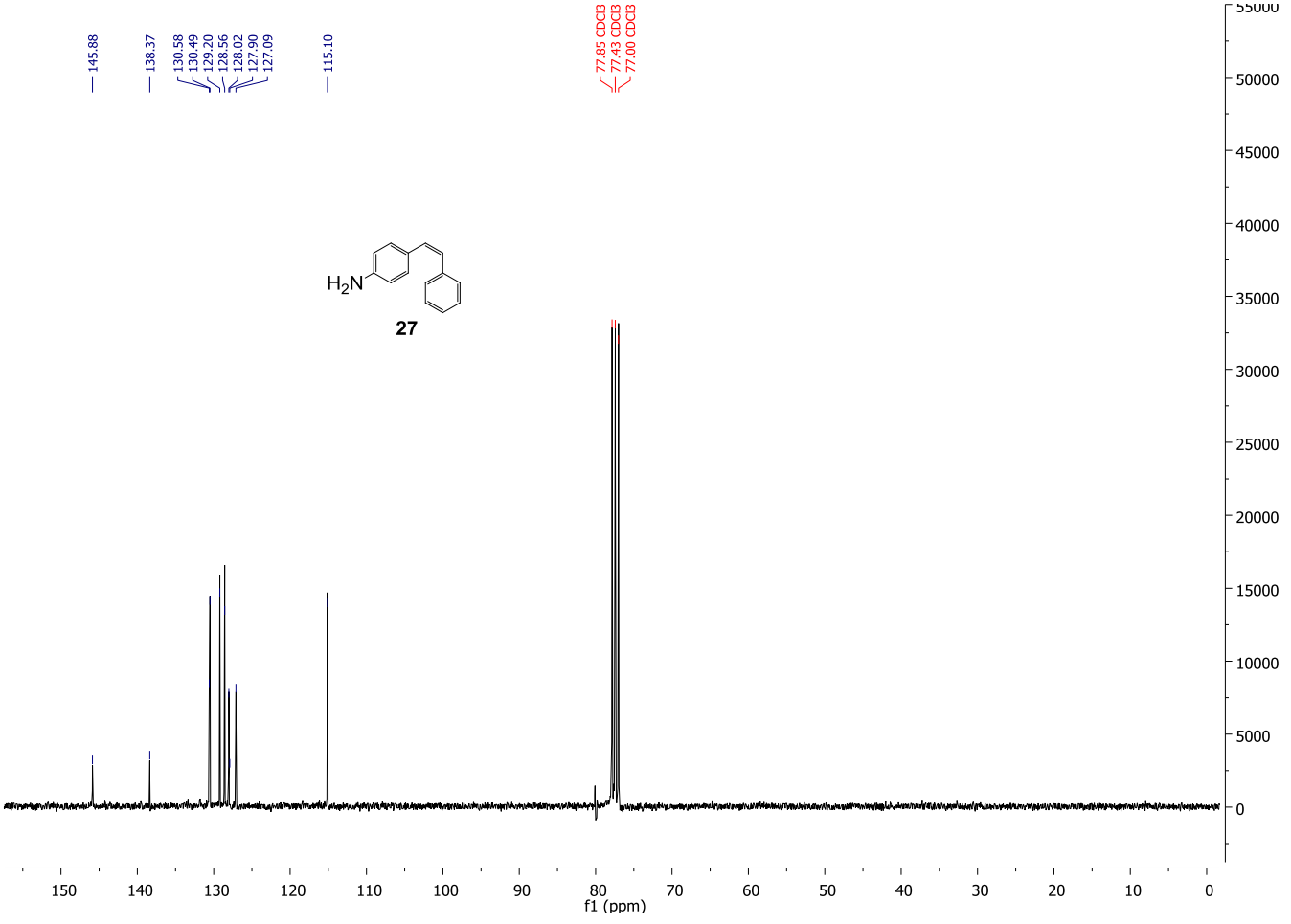

7.26
7.25
7.24
7.24
7.24
7.23
7.22
7.21
7.21
7.19
7.19
7.18
7.17
6.68
6.64
6.58
6.54
1.57
Cl
23
9.20
0.99
1.00
f1 (ppm)

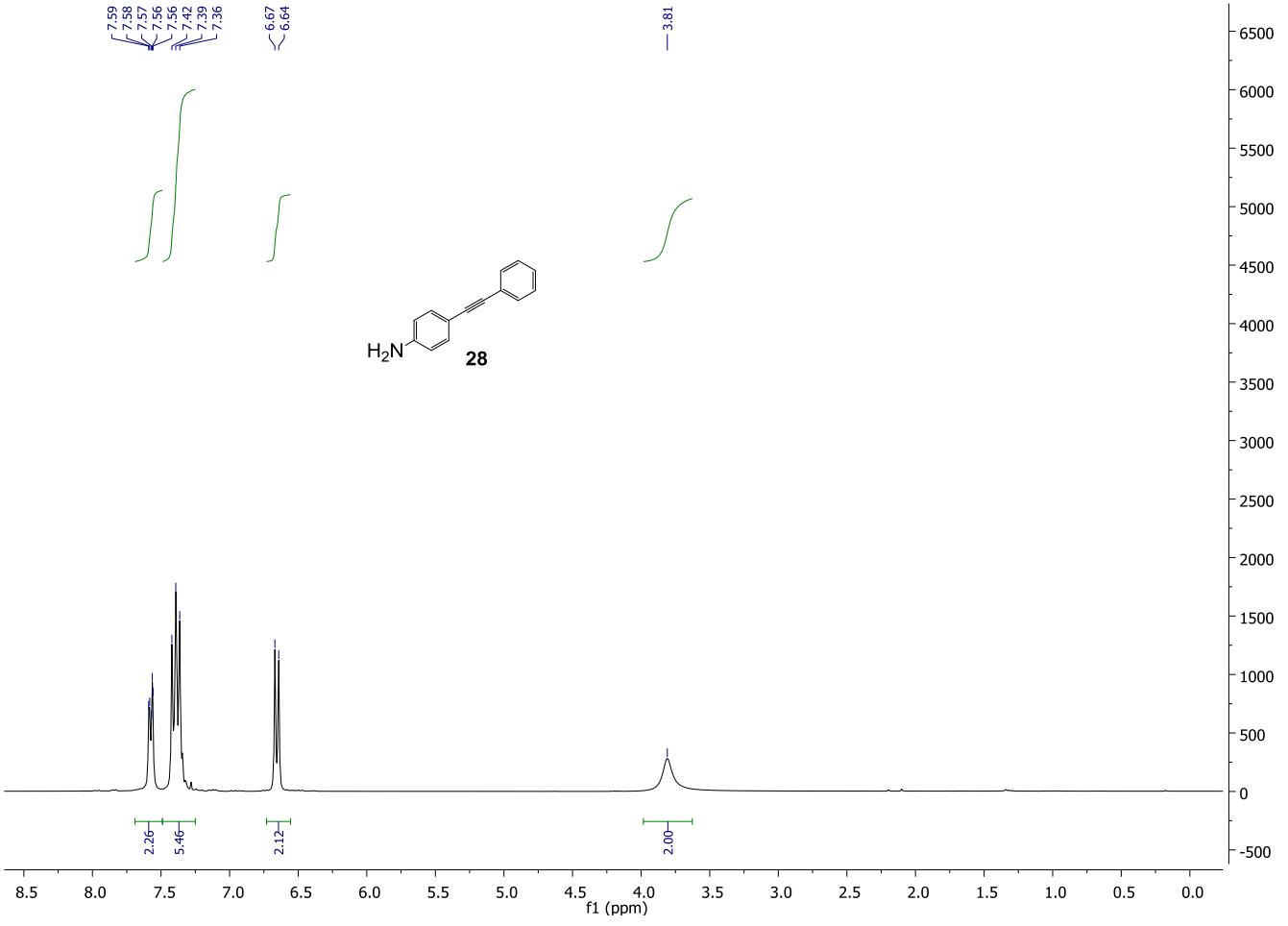

137.26
136.04
133.14
131.35
130.62
129.32
129.21
128.81
128.75
127.73
77.86 CDCl3
77.44 CDCl3
77.02 CDCl3
Cl
23
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
-5000
.50
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

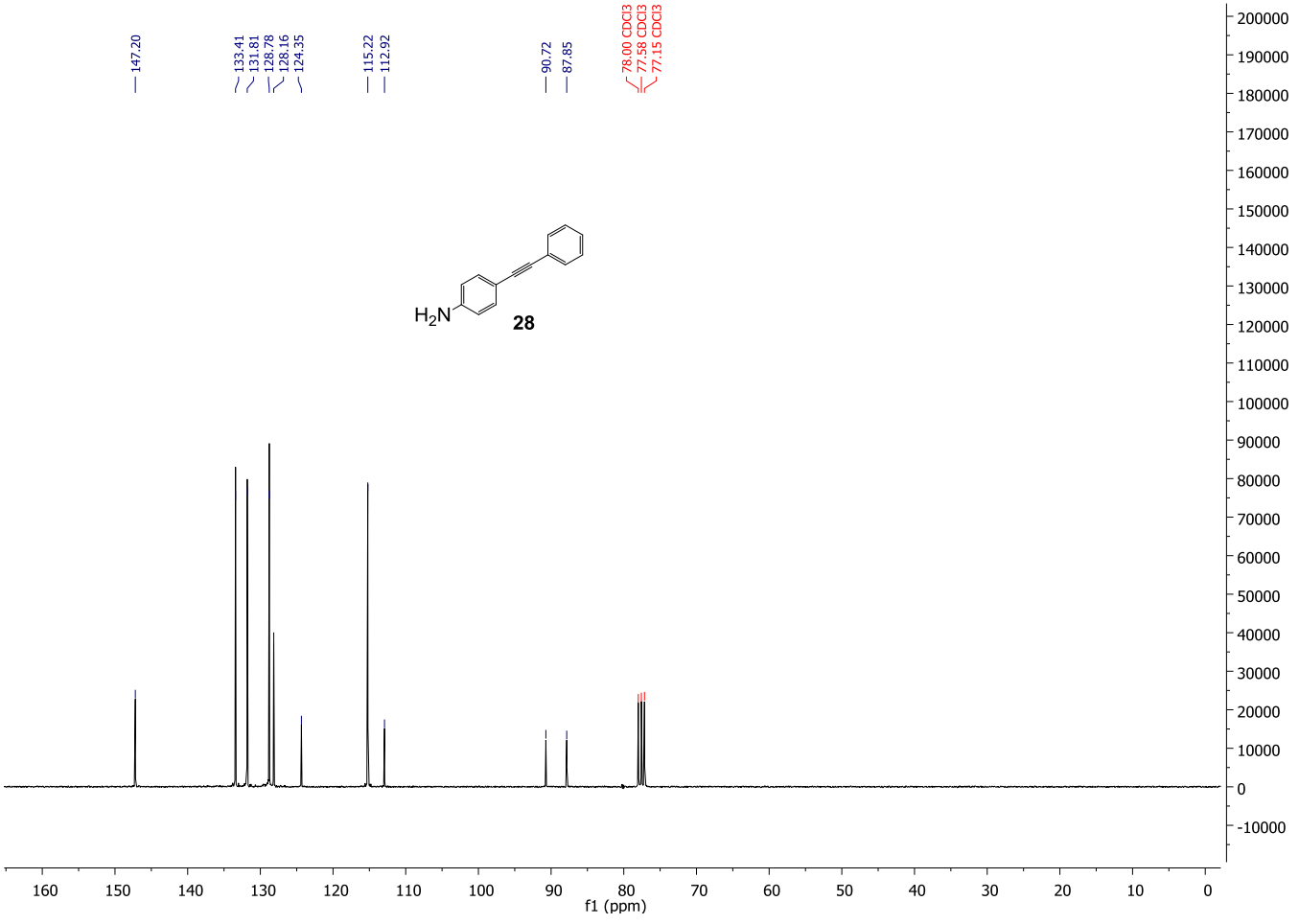

7.85
7.82
7.36
7.33
7.28
7.25
6.77
6.73
6.65
6.61
2.59
1.58
O
25
2.00
2.20
5.66
1.00
1.02
3.00
f1 (ppm)

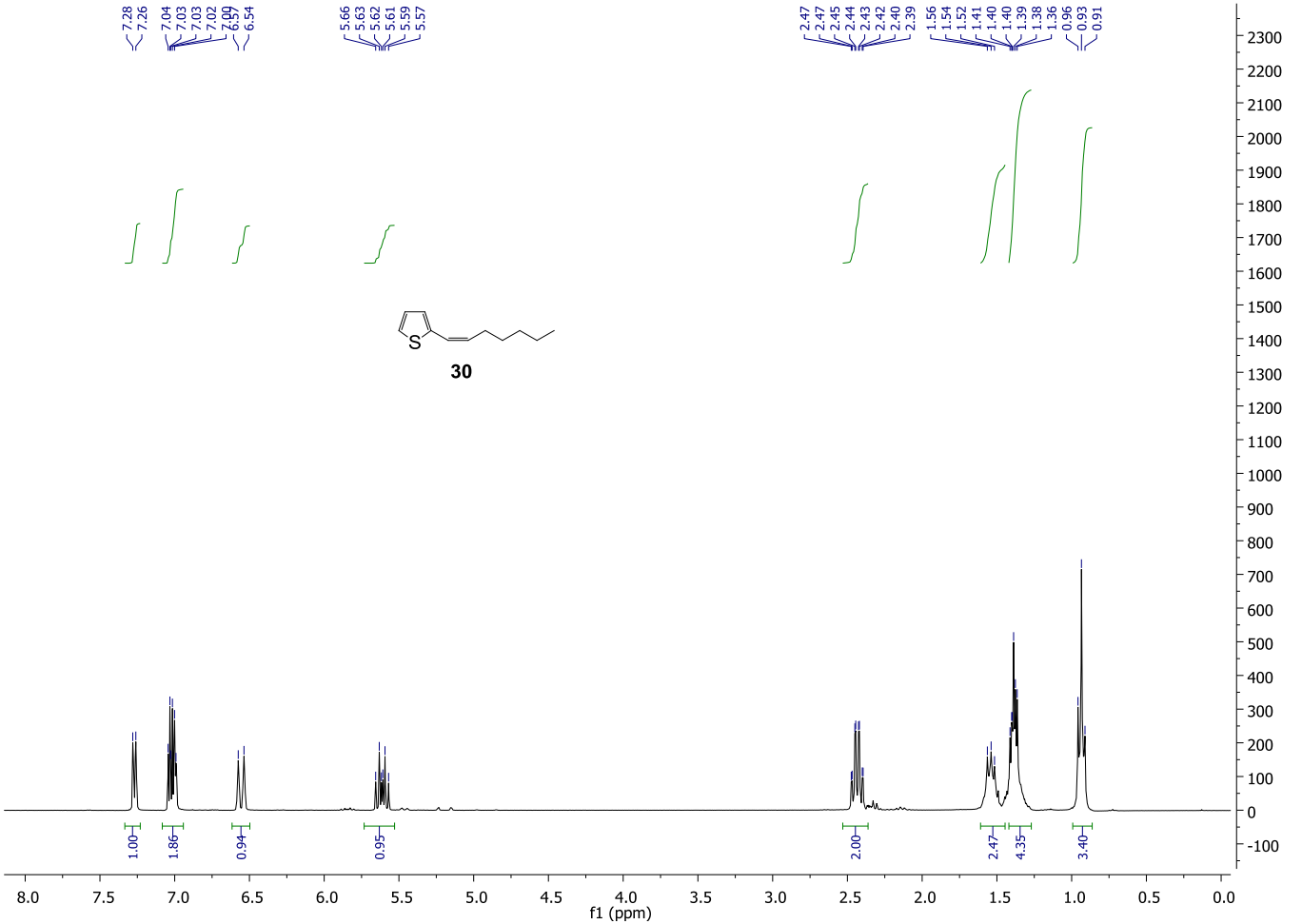

198.07
142.68
137.05
135.98
132.83
129.51
129.45
129.24
128.77
128.73
127.95
77.84 CDCl3
77.00 CDCl3
27.00
O
25
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
-5000
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

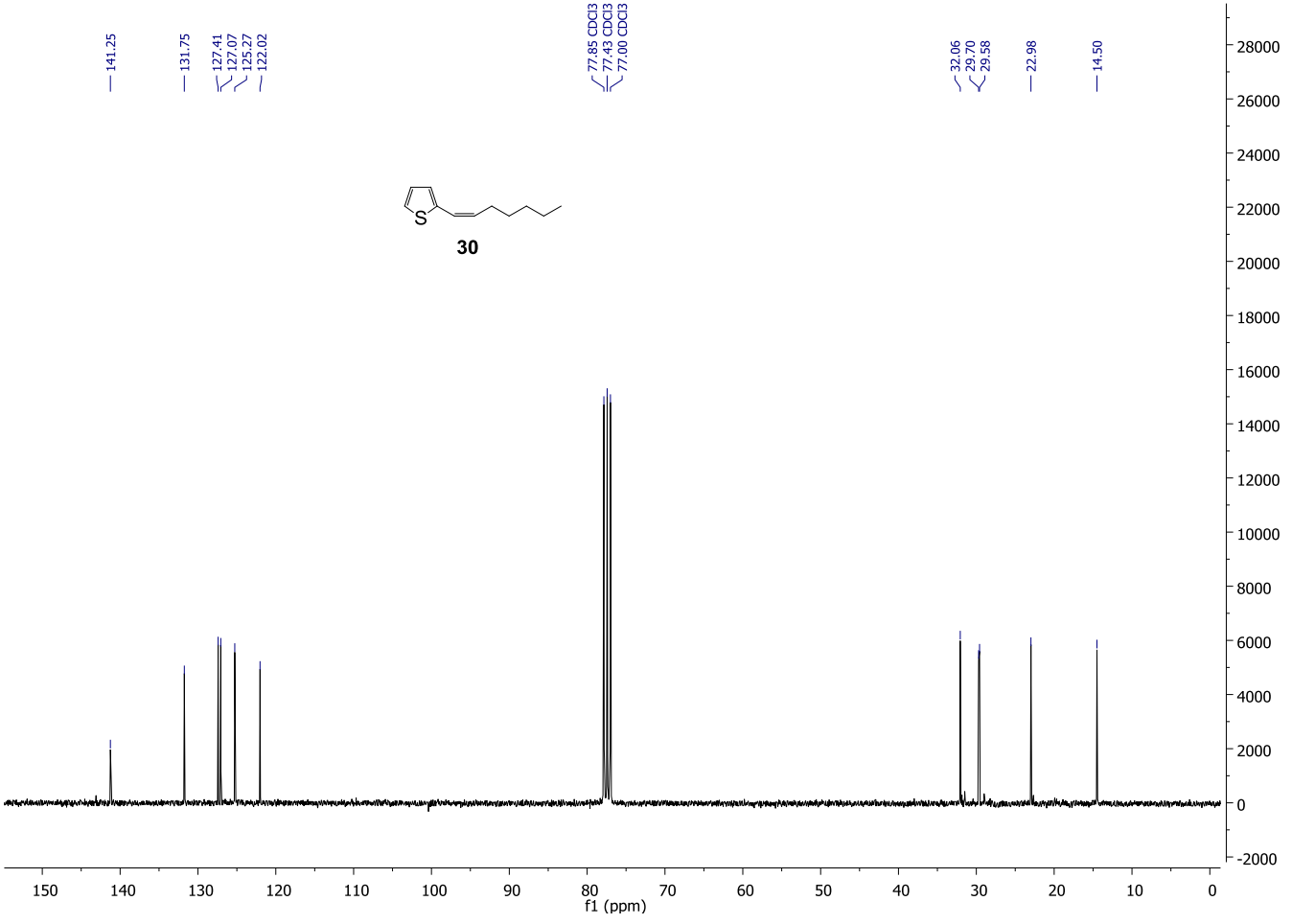

7.34
7.32
7.32
7.31
7.28
7.28
7.26
7.26
7.23
7.11
6.54
6.50
6.48
3.68
H2N
27
5.29
1.91
2.04
1.81
2.00
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

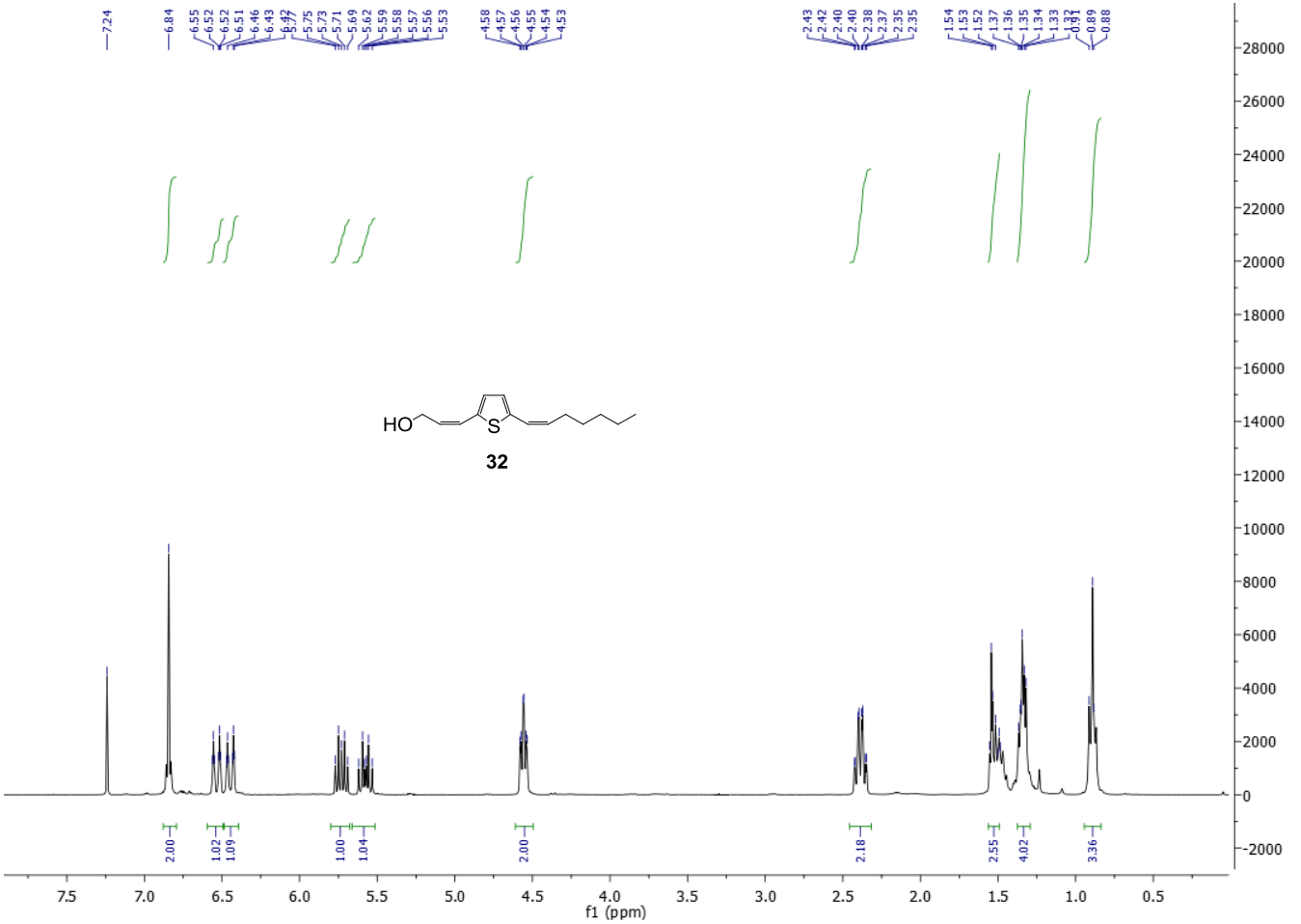

145.88
138.37
130.58
130.49
129.20
128.56
128.02
127.90
127.09
115.10
77.85 CDCl3
77.43 CDCl3
77.00 CDCl3
H2N
27
f1 (ppm)

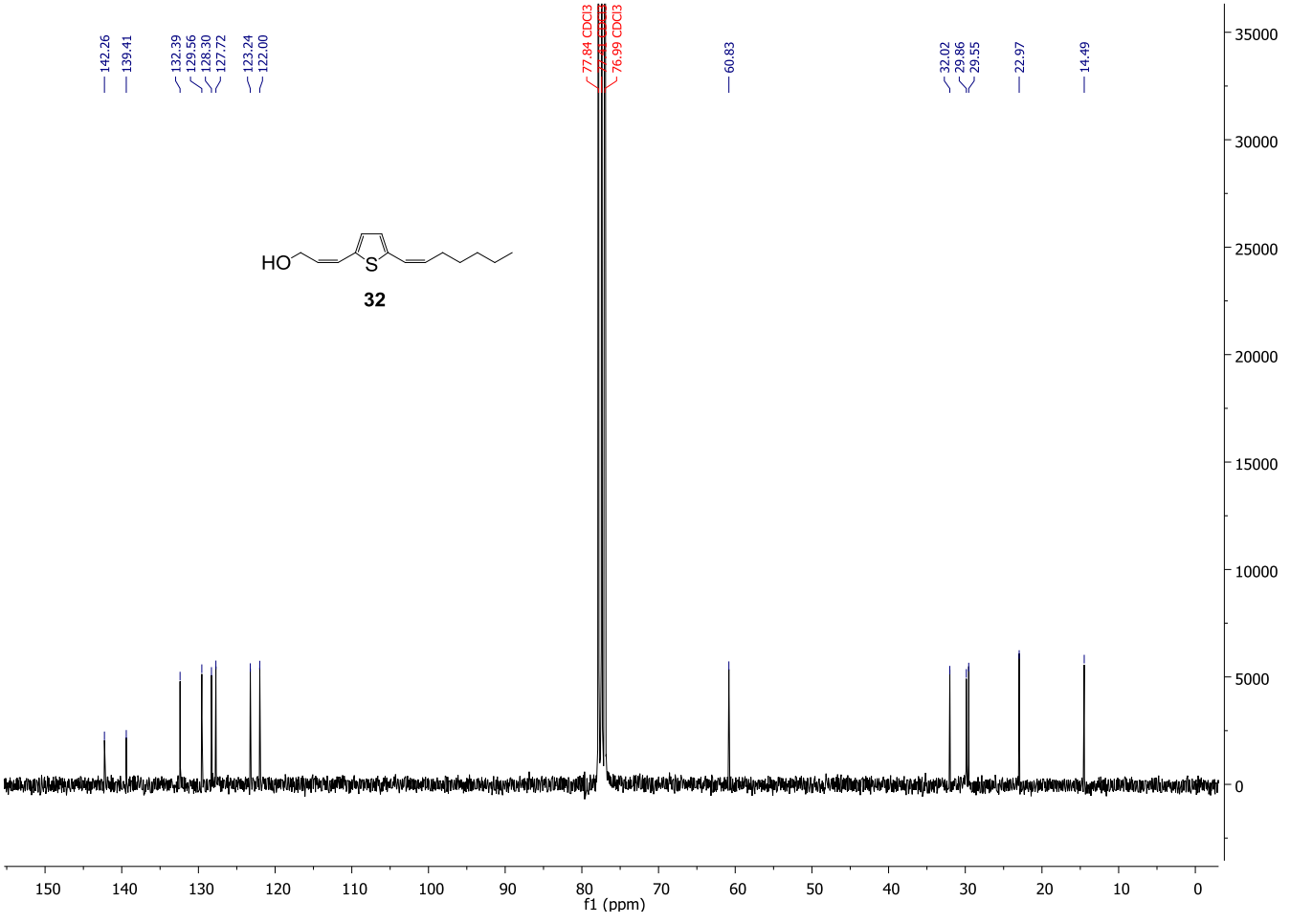

7.59
7.58
7.57
7.56
7.56
7.42
7.39
7.36
6.67
6.64
3.81
H2N
28
2.26
5.46
2.12
2.00
f1 (ppm)


147.20
133.41
131.81
128.78
128.16
124.35
115.22
112.92
90.72
87.85
78.00 CDCl3
77.58 CDCl3
77.15 CDCl3
H2N
28
f1 (ppm)


7.28
7.26
7.04
7.03
7.03
7.02
7.00
6.57
6.54
5.66
5.63
5.62
5.61
5.59
5.57
2.47
2.47
2.45
2.44
2.43
2.42
2.40
2.39
1.56
1.54
1.52
1.41
1.40
1.40
1.39
1.38
1.36
0.96
0.93
0.91
S
30
1.00
1.86
0.94
0.95
2.00
2.47
4.35
3.40
f1 (ppm)

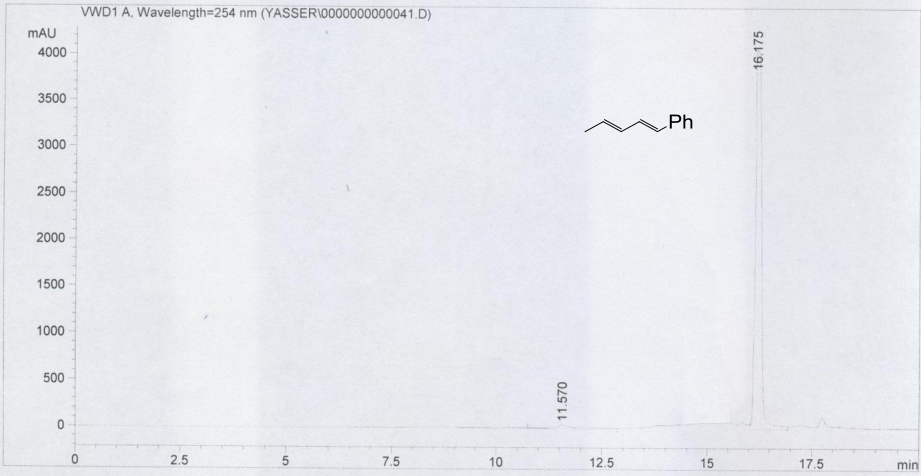

141.25
131.75
127.41
127.07
125.27
122.02
77.85 CDCl3
77.43 CDCl3
77.00 CDCl3
32.06
29.70
29.58
22.98
14.50
S
30
f1 (ppm)


7.24
6.84
6.55
6.52
6.52
6.51
6.46
6.43
5.75
5.73
5.71
5.69
5.62
5.59
5.58
5.57
5.56
5.53
4.58
4.57
4.56
4.55
4.54
4.53
2.43
2.42
2.40
2.40
2.38
2.37
2.35
2.35
1.54
1.53
1.52
1.37
1.36
1.35
1.34
1.33
0.91
0.89
0.88
HO
S
32
2.00
1.02
1.09
1.00
1.04
2.00
2.18
2.55
4.02
3.36
f1 (ppm)


142.26
139.41
132.39
129.56
128.30
127.72
123.24
122.00
77.84 CDCl3
76.99 CDCl3
60.83
32.02
29.86
29.55
22.97
14.49
HO
S
32
35000
30000
25000
20000
15000
10000
5000
0
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

Data File D:\DATA\YASSER\0000000000041.D

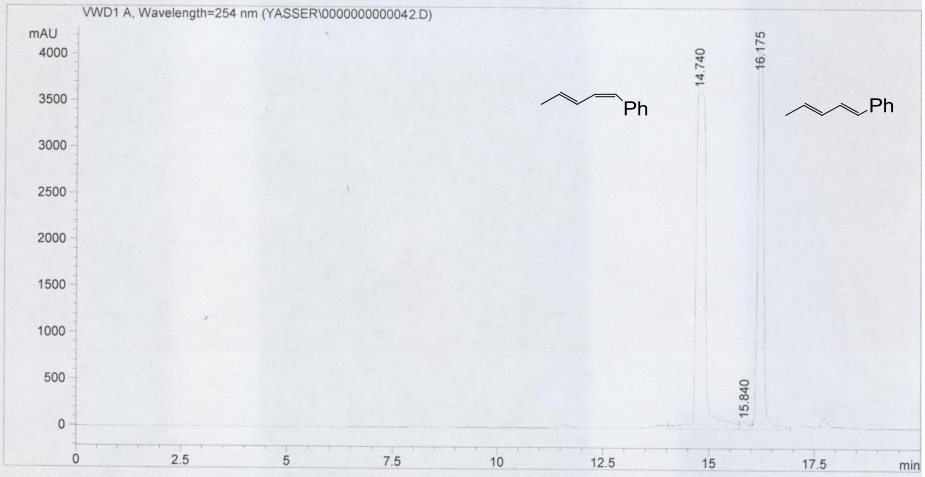
```
=====================================================================
Acq. Operator   : Yasser
Acq. Instrument : Instrument 1                    Location : Vial 1
Injection Date  : 9/3/2012 4:32:45 PM
                                                  Inj Volume : 15 µl
Acq. Method     : D:\METHODS\Yasser\YM-10 13 min-Alex.m
Last changed    : 9/3/2012 4:31:55 PM by Yasser
Analysis Method : D:\METHODS\spalare.m
Last changed    : 9/5/2012 2:55:18 PM by Yasser
                :
```



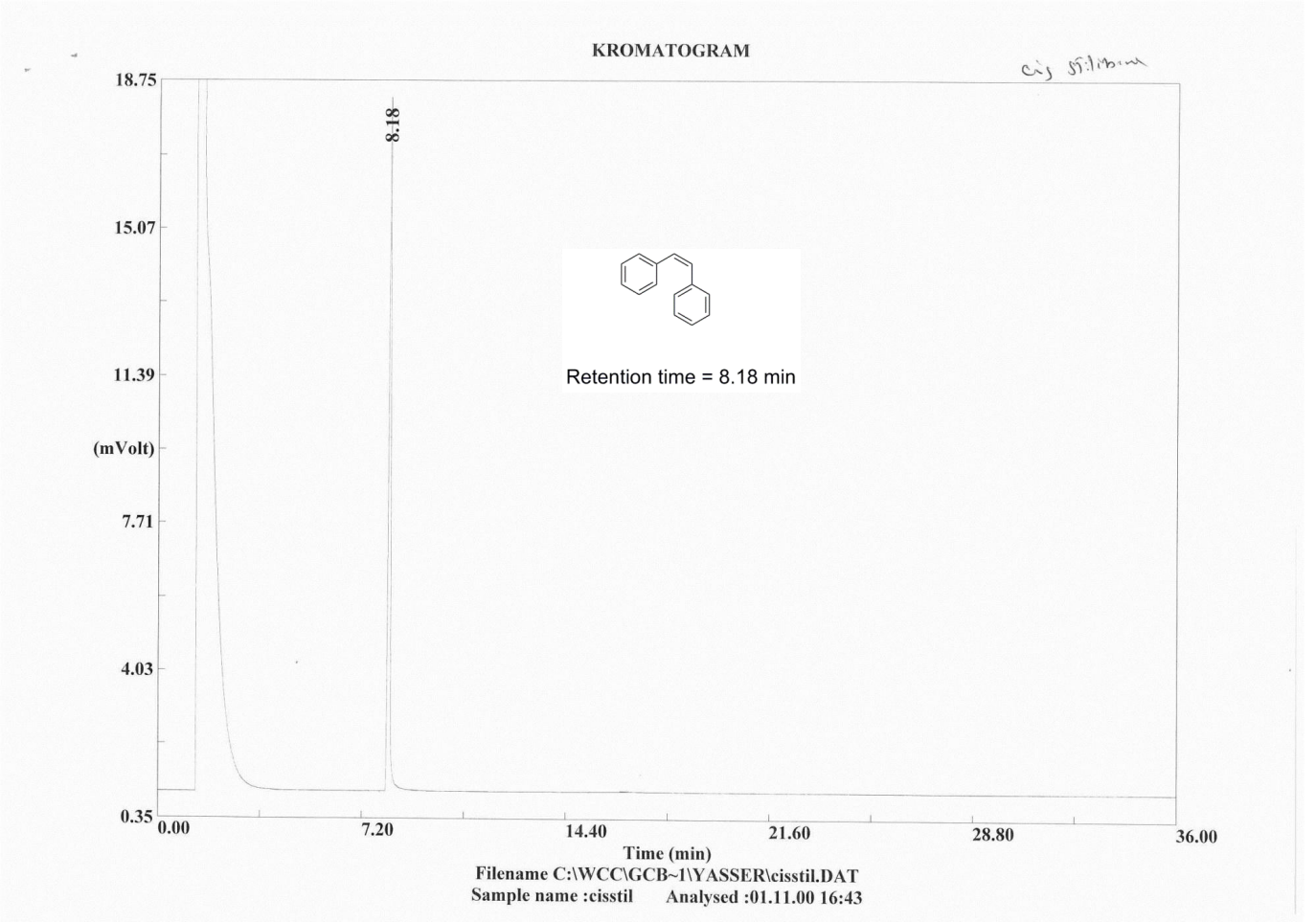

```
=====================================================================
                        Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount         :      1.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=254 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]      [min]  mAU   *s    [mAU   ]      %
----|-------|----|-------|----------|----------|--------|
   1  11.570 VB    0.4342 1224.83228   36.72660   2.7906
   2  16.175 VV    0.1656 4.26673e4  4084.36377  97.2094

Totals :                  4.38921e4  4121.09037

=====================================================================
                        *** End of Report ***


Instrument 1 9/5/2012 3:02:44 PM Yasser
```

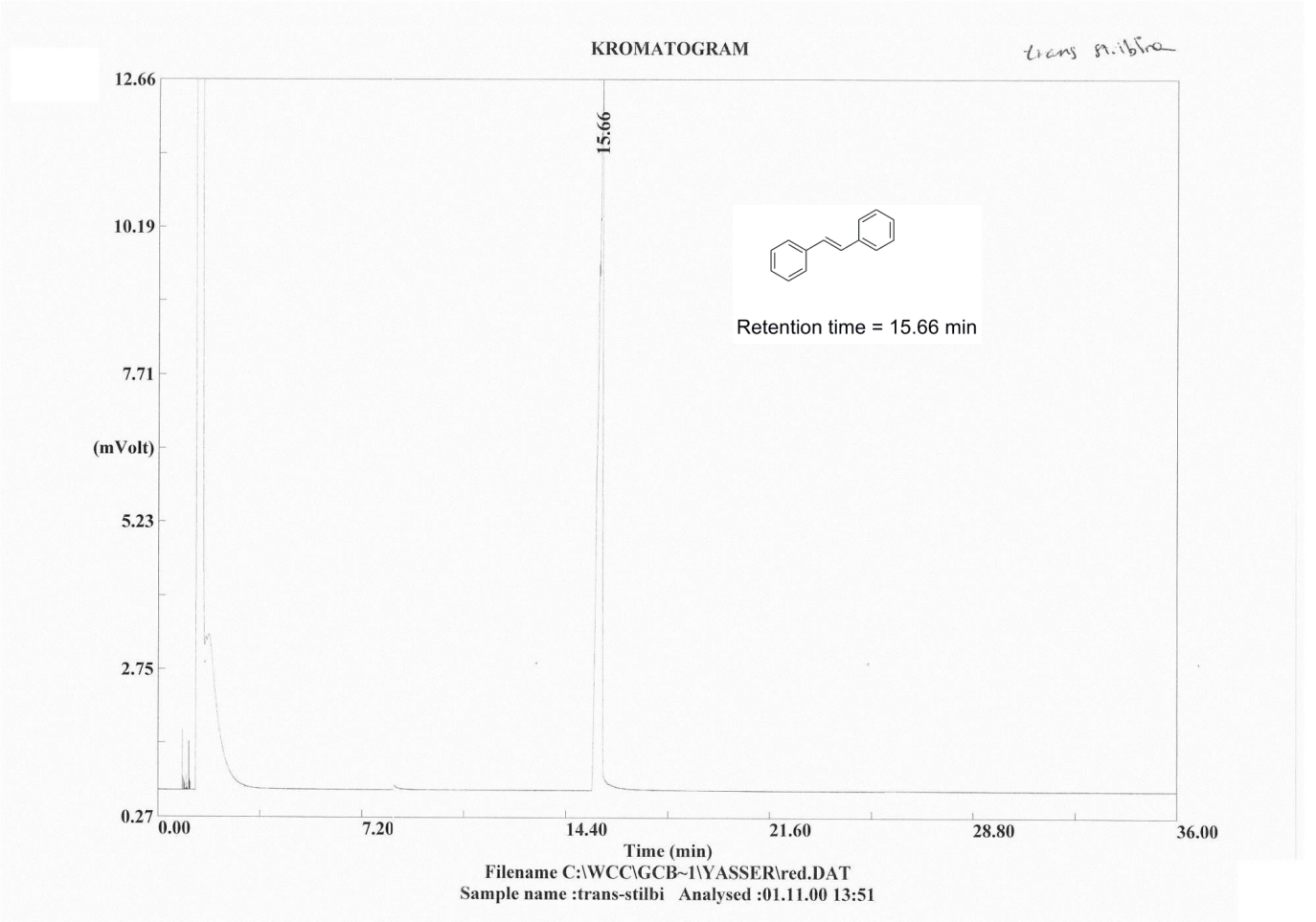
)ata File D:\DATA\YASSER\0000000000042.D

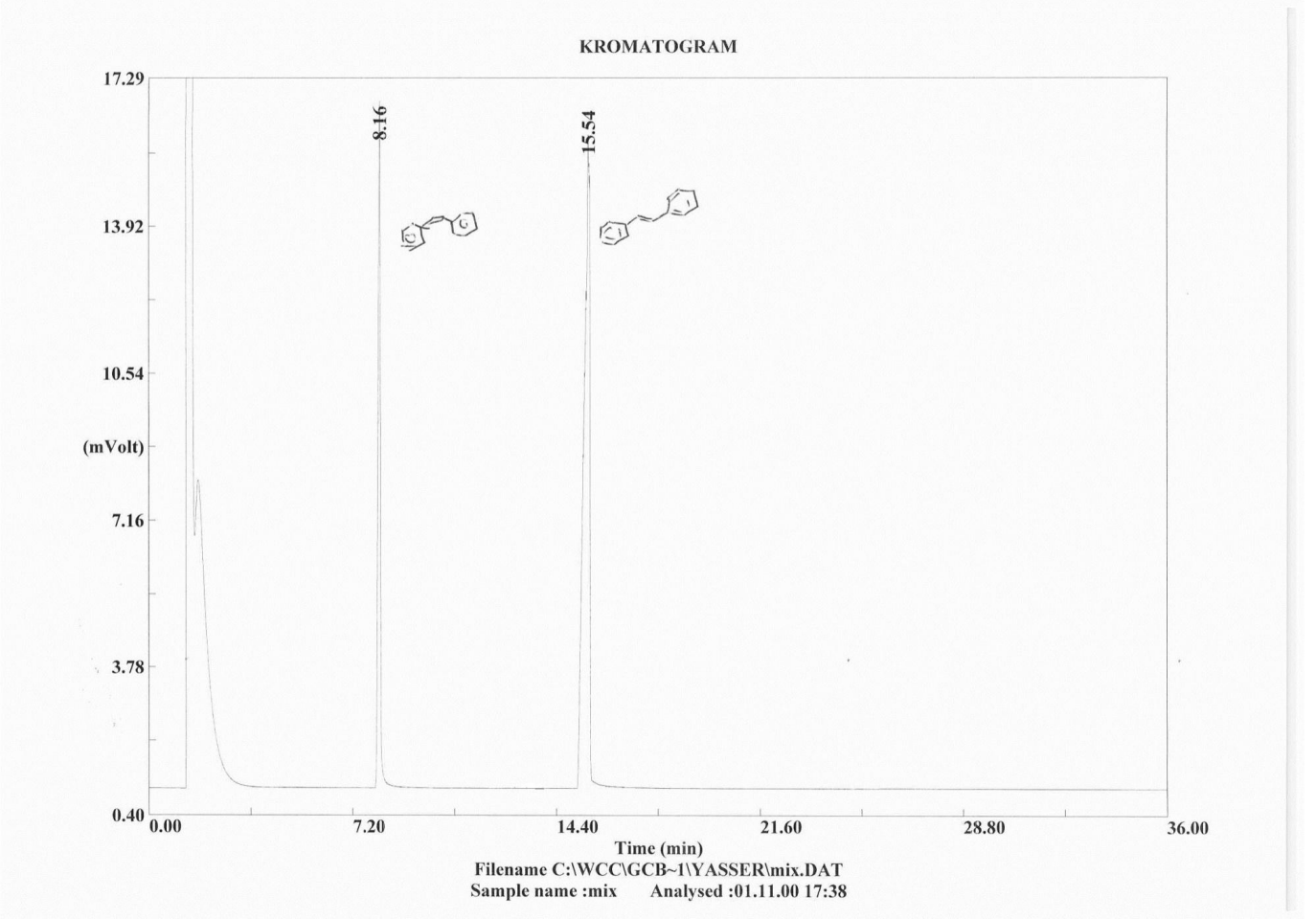
```
=====================================================================
Acq. Operator    : Yasser
Acq. Instrument  : Instrument 1                    Location : Vial 1
Injection Date   : 9/3/2012 4:52:48 PM
                                                   Inj Volume : 15 µl
Acq. Method      : D:\METHODS\Yasser\YM-10 13 min-Alex.m
Last changed     : 9/3/2012 4:51:11 PM by Yasser
Analysis Method  : D:\METHODS\spalare.m
Last changed     : 9/5/2012 2:55:18 PM by Yasser
                 :
```




```
=====================================================================
                        Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount         :      1.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=254 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]       [min]  mAU   *s   [mAU   ]      %
----|-------|----|-------|----------|----------|--------|
   1  14.740 VV    0.2155 5.85632e4  3637.42456  57.2146
   2  15.840 VV    0.1912 1126.63733   79.66655   1.1007
   3  16.175 VV    0.1656 4.26673e4  4084.36377  41.6847

Totals :'                1.02357e5  7801.45488

=====================================================================
                        *** End of Report ***


nstrument 1 9/5/2012 2:56:59 PM Yasser
```


KROMATOGRAM
cis stilbene
18.75
15.07
11.39
(mVolt)
7.71
4.03
0.35
8.18
Retention time = 8.18 min
0.00
7.20
14.40
21.60
28.80
36.00
Time (min)
Filename C:\WCC\GCB~1\YASSER\cisstil.DAT
Sample name :cisstil Analysed :01.11.00 16:43


KROMATOGRAM
trans stilbene
12.66
10.19
7.71
(mVolt)
5.23
2.75
0.27
15.66
Retention time = 15.66 min
0.00
7.20
14.40
21.60
28.80
36.00
Time (min)
Filename C:\WCC\GCB~1\YASSER\red.DAT
Sample name :trans-stilbi Analysed :01.11.00 13:51


KROMATOGRAM
17.29
13.92
10.54
(mVolt)
7.16
3.78
0.40
8.16
15.54
0.00
7.20
14.40
21.60
28.80
36.00
Time (min)
Filename C:\WCC\GCB~1\YASSER\mix.DAT
Sample name :mix Analysed :01.11.00 17:38